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(71) Applicant: GEMSTAR (CAMBRIDGE) LIMITED [GB/GB]; 25A Northgate, Louth, Lincolnshire LN11 0LT (GB).

(72) Inventors: CHATTERJEE, Manash; Biogemma UK Ltd, 200 Science Park, Milton Road, Cambridge CB4 0GZ (GB). BURRELL, Michael, Meyrick; Advanced Technologies (Cambridge) limited, 210 Cambridge Science Park, Cambridge CB4 0WA (GB).

(74) Agent: WALFORD, Margot, Ruth; Patents Department, British American Tobacco R & D Centre, Regents Park Road, Southampton SO15 8TL (GB).

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(54) Title: STARCH MODIFICATION

(57) Abstract: The present invention relates to a method of altering starch synthesis in a plant by modifying the starch priming activity of the plant. In particular, this is achieved by altering the expression or activity of a starch primer which is preferably encoded by the sequence of SEQ ID NO: 1 or a sequence substantially homologous thereto. Also provided are plants in which the starch priming activity has been altered, and propagating material derived from such plants.

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STARCH MODIFICATION

This invention is based upon the identification of a protein, which initiates starch synthesis in a plant. In particular, the intention relates to plant glycogenin-like nucleic acid molecules, plant glycogenin-like gene products, antibodies to plant glycogenin-like gene products, plant glycogenin-like regulatory regions, vectors and expression vectors with plant glycogenin-like genes, cells, plants and plant parts with plant glycogenin-like genes, modified starch from such plants and the use of the foregoing to improve agronomically valuable plants.

Starch, a branched polymer of glucose consisting of largely linear amylose and highly branched amylopectin, is the product of carbon fixation during photosynthesis in plants, and is the primary metabolic energy reserve stored in seeds and fruit. For example, up to 75% of the dry weight of grain in cereals is made up of starch. The importance of starch as a food source is reflected by the fact that two thirds of the world's food consumption (in terms of calories) is provided by the starch in grain crops such as wheat, rice and maize.

Starch is the product of photosynthesis, and is analogous to the storage compound glycogen in eukaryotes. It is produced in the chloroplasts or amyloplasts of plant cells, these being the plastids of photosynthetic cells and non-photosynthetic cells, respectively. The biochemical pathway leading to the production of starch in leaves has been well characterised, and considerable progress has also been made in elucidating the pathway of starch biosynthesis in storage tissues.

The biosynthesis of starch molecules is dependent on a complex interaction of numerous enzymes, including several essential enzymes such as ADP-Glucose, a series of starch synthases which use ADP glucose as a substrate for forming chains of glucose linked by alpha-1-4 linkages, and a series of starch branching enzymes that link sections of polymers with alpha-1-6 linkages to generate branched structures (Smith et al., 1995, Plant Physiology, 107:673-677). Further modification of the starch by yet other enzymes, i.e. debranching enzymes or disproportionating enzymes, can be specific to certain species.

The fine structure of starch is a complex mixture of D-glucose polymers that consist essentially of linear chains (amylose) and branched chains (amylopectin) glucans. Typically, amylose makes up between 10 and 25% of plant starch, but varies significantly among species. Amylose is composed of linear D-glucose chains typically 250-670 glucose units in

length (Tester, 1997, in: Starch Structure and Functionality, Frazier et al., eds., Royal Society of Chemistry, Cambridge, UK). The linear regions of amylopectin are composed of low molecular weight and high molecular weight chains, with the low ranging from 5 to 30 glucose units and the high molecular weight chains from 30 to 100 or more. The amylose/amylopectin ratio and the distribution of low and high molecular weight D-glucose chains can affect starch granule properties such as gelatinization temperature, retrogradation, and viscosity (Blanshard, 1987). The characteristics of the fine structure of starch mentioned above have been examined at length and are well known in the art of starch chemistry.

It is know that starch granule size and amylose percentage change during kernel development in maize and during tobacco leaf development (Boyer et al., 1976, Cereal Chem 53:327-337). In his classic study Boyer et al. concluded the amylose percentage of starch decreases with decreasing granule size in later stages of maize kernel development.

As mentioned above, glycogen serves as the glucose reserve in animals rather than starch. The biosynthesis of glycogen in eukaryotes involves chain elongation through the formation of linear alpha-1,4 glycosidic linkages catalysed by the enzyme, glycogen synthase. Evidence for a distinct initiation step involving a self-glucosylating protein, known as glycogenin or SGP, came from work directed at mammalian systems (Smythe *et al.*, Eur. J. Biochem 200:625-631 (1990) and Whelan Bioessays 5:136-140 (1986)).

Cheng *et al* (Mol. and Cell Biol. 15(12): 6632-6640 (1995)) report the identification of two yeast genes whose products are implicated in the biosynthesis of glycogen. The two genes, Glgl and Glg2 encode self-glucosylating proteins which *in vitro* act as primers for the elongation reaction catalysed by glycogen synthase. Disruption of both these genes results in the inability to synthesise glycogen, despite normal levels of glycogen synthase. Glycogenin homologues have been identified in *Caenorhabditis elegans* and humans (Mu *et al.*, J. Biol. Chem. 272(44): 27589-27597(1997)).

It is now well established that glycogen synthesis is initiated on the primer protein, glycogenin or SGP, which remains covalently attached to the resulting macromolecule. The initiation step is thought to involve glycogenin growing a covalently attached oligosaccharide primer linked via a unique carbohydrate-protein bond via the hydroxyl group of the Tyr residue, Tyr 194. Once this oligosaccharide chain on glycogenin has been extended

sufficiently glycogen synthase is able to catalyse elongation and, together with the branching enzyme, form the mature glycogen molecule (Rodriguez and Whelan, Biochem Biophy Res Comm, 132:829-836; Roach and Skurat, 1997, in Progress in Nucleic Acid Research and Molecular Biology p289-316, Academic Press).

Previous workers have set out to determine whether a priming molecule, such as a self glucosylating protein, is responsible for the initiation of starch synthesis in plants. W094/04693 (Zeneca Ltd.) describes the purification of a putative starch priming protein molecule from maize endosperm, known as amylogenin, and isolation of a partial cDNA. The maize amylogenin showed no sequence homology with glycogenin and exhibited a novel glucose-protein bond (Singh *et al.*, FEBS Letters 376: 61-64 (1995)). However, based upon the sequence homology and the reported properties of the maize protein, it has subsequently been shown that the sequence of the maize nucleic acid molecule reported above is homologous to a reversibly glycosylated polypeptide (RGP1) from pea (Dhugga *et al.*, Proc. Natl.. Acad. Sci. USA 94:7679-7684 (1997)). RGP1 is localised to the Golgi apparatus and is thought to be involved in cell wall synthesis. This has dispelled the initial idea that the "amylogenin" molecule of W094/04693 is involved in starch synthesis. In further work (Langeveld, M.J. S *et al.* 2002 Plant Physiol, 129, pp 278-289) it is concluded that wheat and rice RGPs do not play a role in starch synthesis in a way similar to the functioning of glycogenin as a primer for glycogen synthesis. It is reported that RGP1 and RGP2 proteins in wheat and rice have different functions to glycogenin.

Lightner et al. US 2002/0001843 described fragments of putative "corn (maize), wheat, and rice glycogenin and water stress proteins." Lightner et al. did not demonstrate the functionality of the fragments, but only their sequence homology to glycogenin from animals. To date, therefore, no one has identified and demonstrated a functional protein for starch initiation or starch priming in plants.

Purified starch is used in numerous food and industrial applications and is the major source of carbohydrates in the human diet. Typically, starch is mixed with water and cooked to form a thickening agent or gel. Of central importance are the temperature at which the starch cooks, the viscosity that the agent or gel reaches, and the stability of the gel viscosity over time. The physical properties of unmodified starch limit its usefulness in many

applications. As a result, considerable effort and expenditure is allocated to chemically modify starch (i.e. cross-linking and substitution) in order to overcome the numerous limitations of unmodified starch and to expand industrial usefulness. Modified starches can be used in foods; paper, textiles, and adhesives.

It is an object of the invention to provide novel isolated nucleic acid molecules and isolated polypeptides, which novel molecules and polypeptides are able to provide modified starch properties in transgenically modified plants.

The invention relates to a family of plant glycogenin-like genes, also referred to as starch primer genes. In various embodiments, the invention provides plant glycogenin-like nucleic acid molecules including, but not limited to, plant glycogenin-like genes; plant glycogenin-like regulatory regions; plant glycogenin-like promoters; and vectors incorporating sequences encoding plant glycogenin-like nucleic acid molecules of the invention. Also provided are plant glycogenin-like gene products, including, but not limited to, transcriptional products such as mRNAs, antisense and ribozyme molecules, and translational products such as the plant glycogenin-like protein, polypeptides, peptides and fusion proteins related thereto; genetically engineered host cells that contain any of the foregoing nucleic acid molecules and/or coding sequences or compliments, variants, or fragments thereof operatively associated with a regulatory element that directs the expression of the gene and/or coding sequences in the host cell; genetically-engineered plants derived from host cells; modified starch and starch granules produced by genetically-engineered host cells and plants; and the use of the foregoing to improve agronomically valuable plants.

In the context of the present invention, a "starch primer" used interchangeably with "plant glycogenin-like protein" includes any protein which is capable of initiating starch production in a plant. By definition, the plant glycogenin-like protein will be of plant origin. Preferred fragments of plant glycogenin-like proteins are those which retain the ability to initiate starch synthesis.

The invention is based upon the identification of a protein responsible for initiation of starch synthesis in plants, which despite continued efforts over the last few years, no one had yet successfully identified. In particular, the inventors have discovered nucleic acid molecules from *Arabidopsis* which have sequences that are homologous to the known

glycogenin genes of yeast and human. Analysis of one of this nucleic acid molecule indicates that it contains a sequence encoding a transit peptide for plastid localization of the gene product, consistent with a role in starch synthesis, referred to herein as plant glycogenin-like starch initiation protein (PGSIP). Glycogenin-like genes from other plant species have been identified by analysis of sequence homology with the *Arabidopsis* sequences. The genes of the invention do not show homology to the amylogenin sequences or starch sequences of the prior art.

Modulation of the initiation of starch synthesis allows various aspects of the biosynthetic process to be regulated. By altering aspects of the biosynthesis process such as temporal and spatial specificity, yield and storage, the carbohydrate profile of the plant may be altered in magnitude and directions that may be more favorable for nutritional or industrial uses.

The present invention provides an isolated nucleic acid molecule that i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, or a fragment thereof; ii) comprises a nucleotide sequence at least 40% identical to SEQ ID NOs: 1 or 2, or a complement thereof as determined using the BESTFIT or GAP programs with a gap weight of 50 and a length weight of 3; or iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 1 or 2 under low stringency conditions of hybridization of washing at 60°C for 2x 15 minutes at 2 x SSC, 0.5x SDS, or a complement thereof. The present invention also provides an isolated nucleic acid molecule of the invention comprising SEQ ID NOs: 1 or 2 or a complement thereof. In an embodiment of the invention, an isolated nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of nucleotide residues 516-592, 681-918, 1039-1655, 1762-2536 and 2991-3264 of SEQ ID NO: 1.

Another embodiment of the invention encompasses an isolated nucleic acid molecule of the invention that i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 11, or a fragment thereof; ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NO: 10, or a complement thereof as determined using the BESTFIT or GAP programs with a gap weight of 50 and a length weight of 3, wherein the nucleotide sequence does not encode an amino acid of SEQ ID NO:

35; or iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 10 under stringent conditions of hybridization, or a complement thereof, wherein the sequence does not encode an amino acid of SEQ ID NO: 35. In a related embodiment, the isolated nucleic acid molecule of the invention comprises SEQ ID NO: 10 or a complement thereof. In another related embodiment an isolated nucleic acid molecule of the invention comprises the amino acid sequence that is at least 98% identical to SEQ ID NO: 9 as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4. The invention also encompasses an isolated nucleic acid molecule that comprises the nucleotide sequence of SEQ ID NO: 8 or a complement thereof.

In an embodiment of the invention, an isolated nucleic acid molecule of the invention i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof; ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof as determined using the BESTFIT or GAP programs with a gap weight of 50 and a length weight of 3; or iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33 under stringent conditions of hybridization, or a complement thereof. In a related embodiment, the isolated nucleic acid molecule of the invention comprises SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof. In another embodiment of the invention, a fragment of the isolated nucleic acid molecule of the invention comprises at least 40, 60, 80, 100 or 150 contiguous nucleotides of the nucleic acid molecule. In yet another embodiment, the isolated nucleic acid molecule of the invention comprises the nucleotide sequence of nucleotides 1-195 of SEQ ID NO: 2, or a complement thereof.

According to one aspect of the invention, an isolated polypeptide of the invention comprises the amino acid sequence of amino acid residues 1-65 of SEQ ID NO: 3, or a fragment thereof. In a related aspect, an isolated polypeptide comprises i) an amino acid sequence that is at least 70% identical to SEQ ID NO: 3 or a fragment thereof as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4; ii) an amino acid sequence encoded by the nucleic acid molecule of the invention; or iii) an amino

acid sequence of SEQ ID NO: 3.

An embodiment of the invention encompasses an isolated polypeptide of the invention that comprises i) an amino acid sequence at least 70% identical to SEQ ID NO: 11 as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4, or a fragment thereof; ii) an amino acid sequence encoded by the nucleic acid molecule of of the invention; or iii) an amino acid sequence of SEQ ID NO: 11.

In another embodiment of the invention, an isolated polypeptide of the invention comprises i) an amino acid sequence that is at least 98% identical to SEQ ID NO: 9 as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4; iii) an amino acid sequence encoded by the nucleic acid molecule of SEQ ID NO: 8, or a complement thereof; or v) an amino acid sequence of SEQ ID NO: 9, or a fragment thereof.

The invention further provides for an isolated polypeptide that comprises i) an amino acid sequence that is at least 70% identical to SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof as determined using the BESTFIT or GAP programs with a gap weight of 12 and a length weight of 4; ii) an amino acid sequence encoded by the nucleic acid molecule of the invention; or iii) an amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34. In an embodiment of the invention, a fragment of a polypeptide of the invention comprises at least 5 amino acid residues, wherein said fragment is a portion of the polypeptide encoded by a nucleic acid molecule selected from the group consisting of exon I, exon II, exon III, exon IV and exon V of SEQ ID NO: 1.

Another embodiment of the invention encompasses the polypeptide of SEQ ID: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34 further comprising one or more conservative amino acid substitution. In yet another embodiment, the invention provides for a fusion protein comprising the amino acid sequence of the invention and a heterologous protein.

The invention provides for an isolated polypeptide fragment or immunogenic fragment that comprises at least 5, 8, 10, 15, 20, 25, 30 or 35 consecutive amino acids of a polypeptide according to the invention. The invention further provides for an antibody that immunospecifically binds to a polypeptide of the invention.

In one embodiment the invention encompasses a method for making a polypeptide of any one of the invention, comprising the steps of a) culturing a cell comprising a recombinant polynucleotide encoding a polypeptide of the invention under conditions that allow said polypeptide to be expressed by said cell; and b) recovering the expressed polypeptide.

According to another aspect of the invention, the present invention provides a complex comprising a polypeptide encoded by a nucleic acid molecule of the invention and a starch molecule. In one embodiment of the complex of the invention, the starch molecule comprises from 1 to 700 glucose units. In another embodiment of the complex of the invention the starch molecule comprises branching chains of glucose polysaccharides.

According to yet another aspect of the invention, the present invention provides a vector comprises a nucleic acid molecule of the invention. Alternatively, the present invention provides an expression vector comprises a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule.

Advantageously the expression vector of the invention comprises a regulatory region that confers chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, and/or tissue-specific expression of the nucleic acid molecule or constitutive expression of the nucleic acid molecule of the invention. Advantageously the expression vector of the invention comprises a regulatory region selected from the group consisting of a 35S CaMV promoter, a rice actin promoter, a patatin promoter and a high molecular weight glutenin gene of wheat. In another embodiment, an expression vector of the invention comprises the antisense sequence of a nucleic acid molecule of the invention, wherein the antisense sequence is operably linked to at least one regulatory region.

The invention also provides for a genetically-engineered cell which comprises a nucleic acid molecule of the invention. In one embodiment, a cell comprises the expression vector of the invention comprising a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule. In another embodiment, a cell comprises the expression vector of the invention comprising the antisense sequence of nucleic acid molecules of the invention, wherein the antisense sequence is operably linked to at least one regulatory region.

Yet another aspect of the invention provides a genetically-engineered plant comprising the isolated nucleic acid molecule of the invention. The invention also provides a genetically-engineered plant comprising an isolated nucleic acid molecule of the invention and progeny thereof, and further comprising a transgene encoding an antisense nucleotide sequence. The invention also provides for a genetically-engineered plant comprising an isolated nucleic acid molecule of the invention, and further comprising an RNA interference construct.

An embodiment of the invention encompasses a cell comprising a 35SCaMV constitutive promoter operably linked to a nucleic acid molecule of the invention, fragments thereof, or the nucleic acid molecule of SEQ ID NO:2 or a rice actin promoter operably linked to an RNA interference construct comprising a nucelic acid molecule of the invention, fragments thereof, or fragments of a nucleic acid molecule of SEQ ID NO:2.

Another aspect of the invention provides a method of altering starch synthesis in a plant comprising, introducing into a plant an expression vector of the invention, such that starch synthesis is altered relative to a plant without the expression vector. Yet another embodiment of the invention provides a method of altering starch synthesis in a plant comprising, introducing into a plant at least an expression vector comprising the antisense sequence of a nucleic acid molecules of the invention, wherein the antisense sequence is operably linked to at least one regulatory region, such that starch synthesis is altered in comparison to a plant without the expression vector.

In another aspect of the invention, the present invention provides a method of altering starch granules in a plant comprises introducing into a plant at least an expression vector comprising a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule, such that the starch granules are altered in comparison to a plant without the expression vector.

Advantageously the present invention provides a method of altering starch granules in a plant comprises introducing into a plant at least an expression vector of Claim 30??check, such that the starch granules are altered in comparison to a plant without the expression vector.

The invention further provides a method of altering starch granules in a plant

comprises introducing into a plant at least an expression vector comprising a nucleic acid molecule of the invention and at least one regulatory region operably linked to the nucleic acid molecule, such that the starch granules are absent from leaves of the plant comprising at least an expression vector.

In a preferred embodiment of the invention, a plant part comprises a nucleic acid molecule of the invention resulting in an alteration in starch synthesis. In another preferred embodiment the plant part is a tuber, seed, or leaf.

The invention also provides for the modified starch obtained from the plant parts of the invention, wherein the modification is selected from the group consisting of a ratio of amylose to amylopectin, amylose content, size of starch granules, quantity of size of starch granules, a ratio of small to large starch granules, and rheological properties of the starch as measured using viscometric analysis.

The present invention will now be illustrated by way of non-limiting examples, with reference to the sequence identifiers and Figures in which:

SEQ ID NO:1 shows the genomic sequence of a starch primer gene isolated from *Arabidopsis thaliana* referred to herein as plant glycogenin-like starch initiation protein (PGSIP), at3g18660, GenBank Accession No. NM_112752. The gene includes part of the promoter region, where the putative TATA and CAAT box are located at nucleotides 424-428 and 373-376 respectively. The exons are located at nucleotides 516-592, 681-918, 1039-1655, 1762-2536 and 2991-3264.

SEQ ID NO: 2 shows the deduced cDNA sequence of *Arabidopsis thaliana* PGSIP with protein translation. The transit peptide is located at nucleotides 1-195.

SEQ ID NO:3 shows the amino acid sequence representing the *Arabidopsis thaliana* PGSIP protein. The predicted transit peptide is located at amino acid residues 1-65.

SEQ ID NO:4 shows the nucleotide sequence of the maize EST of GenBank Accession No. BF729544 with homology to the *Arabidopsis thaliana* PGSIP gene. The nucleotide sequence with homology to the *Arabidopsis thaliana* PGSIP gene is located at nucleotides 1-557.

SEQ ID NO:5 shows the nucleotide sequence of the maize EST BG837930 with homology to *Arabidopsis thaliana* PGSIP gene. The nucleotide sequence with homology to the *Arabidopsis thaliana* PGSIP gene is located at nucleotides 1-726.

SEQ ID NO:6 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at1g77130) with protein translation. The protein sequence with homology to a small region (amino acid residues 1023-1146) of dull1 gene from maize (064923).

SEQ ID NO:7 shows the amino acid sequence of at1g77130.

SEQ ID NO:8 shows the deduced cDNA of the Arabidopsis glycogenin-like gene (at1g08990) GenBank Accession No. NM_100770 with protein translation.

SEQ ID NO:9 shows the amino acid sequence of at1g08990.

SEQ ID NO:10 shows the deduced cDNA of the Arabidopsis glycogenin-like gene (at1g54940) GenBank Accession No. NM_104367 with protein translation.

SEQ ID NO:11 shows the amino acid sequence of at1g54940.

SEQ ID NO:12 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at4g33330) GenBank Accession No. NM_119487 with protein translation.

SEQ ID NO:13 shows the amino acid sequence of at4g33330.

SEQ ID NO:14 shows the deduced cDNA of the *Arabidopsis* glycogenin-like gene (at4g33340) GenBank Accession No. NM_119488 with protein translation.

SEQ ID NO:15 shows the amino acid sequence of at4g33340.

SEQ ID No.16 shows the nucleotide sequence of Barley EST Seq1.

SEQ ID NO:17 shows the amino acid sequence of Barley EST Seq1.

SEQ ID NO:18 shows the nucleotide sequence of Barley EST Seq2.

SEQ ID NO:19 shows the amino acid sequence of Barley EST Seq2.

SEQ ID NO:20 shows the nucleotide sequence of a wheat EST.

SEQ ID NO:21 shows the first half of the amino acid sequence of the wheat EST.

SEQ ID NO:22 shows the second half of the amino acid sequence of the wheat EST.

SEQ ID NO:23 shows the deduced cDNA of the *Arabidopsis* gene EMBL:AY062695 GenBank Accession No. AY062695 with homology to the *Arabidopsis* PGSIP gene with protein translation.

SEQ ID NO:24 shows the amino acid sequence of EMBL:AY062695.

SEQ ID NO:25 shows the deduced cDNA of the Rice gene SPTrEMBL:Q94HG3 GenBank Accession No. *AC079633* with homology to the *Arabidopsis* PGSIP gene with protein translation.

SEQ ID NO:26 shows the amino acid sequence of SPTrEMBL:Q94HG3.

SEQ ID NO:27 shows the nucleotide sequence of Maize EST Seq1.

SEQ ID NO:28 shows the amino acid sequence of Maize EST Seq1.

SEQ ID NO:29 shows the nucleotide sequence of Maize EST Seq2.

SEQ ID NO:30 shows the amino acid sequence of Maize EST Seq2.

SEQ ID NO:31 shows the nucleotide sequence of Maize EST Seq3.

SEQ ID NO:32 shows the amino acid sequence of Maize EST Seq3.

SEQ ID NO:33 shows the nucleotide sequence of Maize EST Seq4.

SEQ ID NO: 34 shows the amino acid sequence of Maize EST Seq4.

SEQ ID NO: 35 shows an amino acid sequence as a result of a conceptual translation of a portion of a genomic clone from *Arabidopsis thaliana* as it appears in US Patent Application No. 2002/0001843.

Figure 1 shows the plasmid containing the *Arabidopsis thaliana* plant glycogenin-like starch initiation protein (PGSIP) gene.

Figure 2 shows the plasmid map for pTPYES.

Figure 3 shows the plasmid map for pNTPYES

Figure 4A shows a genomic region containing AT3g18660 (PGSIP); 4B shows a non-radioactive southern blot of *Arabidopsis*, wheat and maize genomic DNA probed with C-terminus AT3g18660 cDNA under high stringency conditions. N-NcoI, A-AvaI, C-ClaI. The probe used for the blot of Figure 4B is also shown.

Figure 5A shows a non-radioactive southern blot of *Arabidopsis*, wheat and maize genomic DNA probed with N-terminal ATg18660 (PGSIP) cDNA fragment under low stringency conditions. N-NcoI, A-AvaI, C-ClaI. Lane M is a marker, lane 1 is AT (EcoRI), lane 2 is AT (XhoI), lane 3 is AT (EcoRV), lane 4 is wheat (EcoRI), lane 5 is wheat (XhoI), lane 6 is wheat EcoRV), lane 7 is maize (EcorRI), lane 8 is maize (XhoI), and lane 9 is maize (EcoRV); 5B shows a non-radioactive southern blot of *Arabidopsis*, wheat and maize genomic DNA probed with C-terminal ATg18660 (PGSIP) cDNA fragment under low stringency conditions. N-NcoI, A-AvaI, C-ClaI. Lane M is a marker, lane 1 is AT (EcoRI), lane 2 is AT (XhoI), lane 3 is AT (EcoRV), lane 4 is wheat (EcoRI), lane 5 is wheat (XhoI),

lane 6 is wheat EcoRV), lane 7 is maize (EcorRI), lane 8 is maize (XhoI), and lane 9 is maize (EcoRV): 5C shows the N-terminal and C-terminal region of the PGSIP cDNA used to probe the blots of 5A and 5B.

Figure 6 shows the cloning strategy and plasmid maps for the production of the PGSIP RNAi construct pCL76 SCV.

Figure 7 shows the plasmid map for pCL68 SCV. (Sense expression construct) containing the AT3g18660 (PGSIP) cDNA.

Figure 8 shows the plasmid map for pCL76 SCV.(RNAi construct) containing fragments of the AT3g18660 (PGSIP) cDNA.

Figure 9 shows the plasmid map for pMC177 (Sense expression construct) containing the AT3g18660 (PGSIP) under rice actin promoter used in barley and *Arabidopsis* transformation.

Figure 10 shows the plasmid map for pMC176 (RNAi construct) containing the AT3g18660 (PGSIP) under rice actin promoter used in barley and *Arabidopsis* transformation.

Figure 11A shows the results of iodine staining of leaves of barley which was shown to be PCR positive for the (pCL76 SCV) RNAi PGSIP constructs. Starch grains are absent; 11B shows the results of iodine staining of leaves of barley which was shown to be PCR negative for the (pCL76 SCV) RNAi PGSIP constructs. Starch grains are visible.

For purposes of clarity, and not by way of limitation, the invention is described in the subsections below in terms of (a) plant glycogenin-like nucleic acid molecules; (b) plant glycogenin-like gene products; (c) transgenic plants that ectopically express plant glycogenin-like protein; (d); transgenic plants in which endogenous plant glycogenin-like protein expression is suppressed; (e) starch characterized by altered structure and physical properties produced by the methods of the invention.

1.0 PLANT GLYCOGENIN-LIKE NUCLEIC ACIDS

The nucleic acid molecules of the invention may be DNA, RNA and comprises the nucleotide sequences of a plant glycogenin-like gene, or fragments or variants thereof. A polynucleotide is intended to include DNA molecules (*e.g.*, cDNA, genomic DNA), RNA molecules (*e.g.*, hnRNA, pre-mRNA, mRNA, double-stranded RNA), and DNA or RNA

analogs generated using nucleotide analogs. The polynucleotide can be single-stranded or double-stranded.

The nucleic acid molecules are characterized by their homology to known glycogen primer (glycogenin) genes, such as those from yeast (Glgl and Glg2), human (any isoform), C. *elegans*, rat or rabbit, or plant glycogenin-like gene such as those defined herein. A preferred nucleic acid molecule of this embodiment is one that encodes the amino acid sequence of SEQ ID NO: 2, or a fragment or variant thereof, or a nucleic acid molecule comprising a sequence substantially similar to SEQ ID NO: 2. In a most preferred embodiment, the nucleic acid molecule comprises the nucleotide sequence shown in SEQ ID NO: 1, or a fragment or variant thereof, or a sequence substantially similar to SEQ ID NO: 1. The variants may be an allelic variants. Allelic variants being multiple forms of a particular gene or protein encoded by a particular gene. Fragments of a plant glycogenin-like gene may include regulatory elements of the gene such as promoters, enhancers, transcription factor binding sites, and/or segments of a coding sequence for example, a conserved domain, exon, or transit peptide.

In a preferred embodiment, the nucleic acid molecules of the invention are comprised of full length sequences in that they encode an entire plant glycogenin-like protein as it occurs in nature. Examples of such sequences include SEQ ID NOs: 1, 2, 6, 8, 10, 12, and 14. The corresponding amino acid sequences of full length glycogenin-like proteins are SEQ ID NOs: 3, 7, 9, 11, 13, and 15.

In an alternative embodiment, the nucleic acid molecules of the invention comprise a nucleotide sequence of SEQ ID NOs: 1, 2, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33.

The nucleic acid molecules and their variants can be identified by several approaches including but not limited to analysis of sequence similarity and hybridization assays.

In the context of the present invention the term "substantially homologous," "substantially identical," or "substantial similarity," when used herein with respect to sequences of nucleic acid molecules, means that the sequence has either at least 45% sequence identity with the reference sequence, preferably 50% sequence identity, more preferably at least 60%, 70%, 80%, 90% and most preferably at least 95% sequence identity

with said sequences, in some cases the sequence identity may be 98% or more preferably 99%, or above, or the term means that the nucleic acid molecule is either is capable of hybridizing to the complement of the nucleic acid molecule having the reference sequence under stringent conditions.

"% identity", as known in the art, is a measure of the relationship between two polynucleotides or two polypeptides, as determined by comparing their sequences. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. The alignment of the two sequences is examined and the number of positions giving an exact amino acid or nucleotide correspondence between the two sequences determined, divided by the total length of the alignment and multiplied by 100 to give a % identity figure. This % identity figure may be determined over the whole length of the sequences to be compared, which is particularly suitable for sequences of the same or very similar length and which are highly homologous, or over shorter defined lengths, which is more suitable for sequences of unequal length or which have a lower level of homology.

For example, sequences can be aligned with the software clustalw under Unix which generates a file with a ".aln" extension, this file can then be imported into the Bioedit program (Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98) which opens the .aln file. In the Bioedit window, one can choose individual sequences (two at a time) and alignment them. This method allows for comparison of the entire sequences.

Methods for comparing the identity of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J *et al*, Nucleic Acids Res. 12:387-395, 1984, available from Genetics Computer Group, Maidson, Wisconsin, USA). The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (Advances in Applied Mathematics, 2:482-489, 1981) and finds the best single region of similarity between two sequences. BESTFIT is more suited to comparing two polynucleotide or two polypeptide sequences

which are dissimilar in length, the program assuming that the shorter sequence represents a portion of the longer. In comparison, GAP aligns two sequences finding a "maximum similarity" according to the algorithm of Neddleman and Wunsch (J. Mol. Biol. 48:443-354, 1970). GAP is more suited to comparing sequences which are approximately the same length and an alignment is expected over the entire length. Preferably the parameters "Gap Weight" and "Length Weight" used in each program are 50 and 3 for polynucleotides and 12 and 4 for polypeptides, respectively. Preferably % identities and similarities are determined when the two sequences being compared are optimally aligned.

Other programs for determining identity and/or similarity between sequences are also known in the art, for instance the BLAST family of programs (Karlin & Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin & Altschul, 1993, *Proc. Natl. Acad. Sci. USA* 90:5873-5877, available from the National Center for Biotechnology Information (NCB), Bethesda, Maryland, USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov). These programs exemplify a preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences. Such an algorithm is incorporated into the BLASTN and BLASTX programs of Altschul, et al., 1990, *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, BLASTX and BLASTN) can be used. *See* http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid

sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Another non-limiting example of a program for determining identity and/or similarity between sequences known in the art is FASTA (Pearson W.R. and Lipman D.J., Proc. Nat. Acac. Sci., USA, 85:2444-2448, 1988, available as part of the Wisconsin Sequence Analysis Package). Preferably the BLOSUM62 amino acid substitution matrix (Henikoff S. and Henikoff J.G., Proc. Nat. Acad. Sci., USA, 89:10915-10919, 1992) is used in polypeptide sequence comparisons including where nucleotide sequences are first translated into amino acid sequences before comparison.

Yet another non-limiting example of a program known in the art for determining identity and/or similarity between amino acid sequences is SeqWeb Software (a web-based interface to the GCG Wisconsin Package: Gap program) which is utilized with the default algorithm and parameter settings of the program: blosum62, gap weight 8, length weight 2.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

Preferably the program BESTFIT is used to determine the % identity of a query polynucleotide or a polypeptide sequence with respect to a polynucleotide or a polypeptide sequence of the present invention, the query and the reference sequence being optimally aligned and the parameters of the program set at the default value.

Alternatively, variants and fragments of the nucleic acid molecules of the invention can be identified by hybridization to SEQ ID NOs: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33. In the context of the present invention "stringent conditions" are defined as those given in Martin *et al* (EMBO J 4:1625-1630 (1985)) and Davies *et al* (Methods in Molecular Biology Vol 28: Protocols for nucleic acid analysis by non-radioactive probes, Isaac, P.G. (ed), Humana Press Inc., Totowa N.J, USA)). Hybridization was carried out overnight at 65°C (high stringency conditions) or 55°C (low stringency conditions). The filters were washed for 2 x 15 minutes with 0.1 x SSC, 0.5 x SDS at 65°C (high stringency washing). For low

stringency washing, the filters were washed at 60°C for 2x 15 minutes at 2 x SSC, 0.5x SDS.

In instances wherein the nucleic acid molecules are oligonucleotides ("oligos"), highly stringent conditions may refer, *e.g.*, to washing in 6xSSC / 0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These nucleic acid molecules may act as plant glycogenin-like gene antisense molecules, useful, for example, in plant glycogenin-like gene regulation and/or as antisense primers in amplification reactions of plant glycogenin-like gene and/or nucleic acid molecules. Further, such nucleic acid molecules may be used as part of ribozyme and/or triple helix sequences, also useful for plant glycogenin-like gene regulation. Still further, such molecules may be used as components in probing methods whereby the presence of a plant glycogenin-like allele may be detected.

In one embodiment, a nucleic acid molecule of the invention may be used to identify other plant glycogenin-like genes by identifying homologs. This procedure may be performed using standard techniques known in the art, for example screening of a cDNA library by probing; amplification of candidate nucleic acid molecules; complementation analysis, and yeast two-hybrid system (Fields and Song Nature 340 245-246 (1989); Green and Hannah Plant Cell 10 1295-1306 (1998)).

The invention also includes nucleic acid molecules, preferably DNA molecules, that are amplified using the polymerase chain reaction and that encode a gene product functionally equivalent to a plant glycogenin-like gene product.

In another embodiment of the invention, nucleic acid molecules which hybridize under stringent conditions to the nucleic acid molecules comprising a plant glycogenin-like gene and its complement are used in altering starch synthesis in a plant. Such nucleic acid molecules may hybridize to any part of a plant glycogenin-like gene, including the regulatory elements. Preferred nucleic acid molecules are those which hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence encoding the amino acid sequence of SE ID NO: 2, and/or a nucleotide sequence of any one of SEQ ID NOs: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33 or their complement sequences. Preferably the nucleic acid molecule which hybridizes under stringent conditions to a nucleic acid molecule comprising the sequence of a plant glycogenin-like gene or its complement are

complementary to the nucleic acid molecule to which they hybridize.

In another embodiment of the invention, nucleic acid molecules which hybridize under stringent conditions to the nucleic acid molecules of SEQ ID NOs: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, or 33 hybridize over the full length of the sequences of the nucleic acid molecules.

Alternatively, nucleic acid molecules of the invention or their expression products may be used in screening for agents which alter the activity of a plant glycogenin-like protein of a plant. Such a screen will typically comprise contacting a putative agent with a nucleic acid molecule of the invention or expression product thereof and monitoring the reaction there between. The reaction may be monitored by expression of a reporter gene operably linked to a nucleic acid molecule of the invention, or by binding assays which will be known to persons skilled in the art.

Fragments of a plant glycogenin-like nucleic acid molecule of the invention preferably comprise or consist of at least 40 continuous or consecutive nucleotides of the plant glycogenin-like nucleic acid molecule of the invention, more preferably at least 60 nucleotides, at least 80 nucleotides, or most preferably at least 100 or 150 nucleotides in length. Fragments of a plant glycogenin-like nucleic acid molecule of the invention encompassed by the invention may include elements involved in regulating expression of the gene or may encode functional plant glycogenin-like proteins. Fragments of the nucleic acid molecules of the invention, encompasses fragments of SEQ ID NOs: 1, 2, 4-6, 8, 10, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31 and 33 as well as fragments of the variants of those sequences identified as defined above by percent homology or hybridization.

Examples of fragments encompassed by the invention include exons of the PGSIP gene. SEQ ID NO: 1 indicates exon and intron boundaries of the plant glycogenin-like gene PGSIP. Nucleic acid molecules comprising PGSIP exon and intron sequences are encompassed by the present invention. In one embodiment, five exons are included (SEQ ID NO:1; GenBank Accession No. NM_112752). PGSIP exon 1 encompasses nucleotides 516-592 of SEQ ID NO: 1. of the sequence shown in SEQ ID NO:1; exon 2 encompasses nucleotides 681 to 918 of the sequence shown in SEQ ID NO:1; exon 3 encompasses nucleotides 1039 to 1655 of the sequence shown in SEQ ID NO:1; exon 4 encompasses

nucleotides 1762 to 2536 of the sequence shown in SEQ ID NO:1; exon 5 encompasses nucleotides 2991 to 3264 of the sequence shown in SEQ ID NO:1.

Further, a plant glycogenin-like nucleic acid molecule of the invention can comprise two or more of any above-described sequences, or variants thereof, linked together to form a larger subsequence.

The nucleic acid molecules of the invention can comprise or consist of an EST sequence. The EST nucleic acid molecules of the invention can be used as probes for cloning corresponding full length genes. For example, the barley EST of SEQ ID NO: 16 can be utilized as a probe in identifying and cloning the full length Barley homolog of the *Arabidopsis* PGSIP gene. The EST nucleic acid molecules of the invention may be used as sequence probes in connection with computer software to search databases, such as GenBank for homologous sequences. Alternatively, the EST nucleic acid molecules can be used as probes in hybridization reactions as described herein. The EST nucleic acid molecules of the invention can also be used as molecular markers to map chromosome regions.

In certain embodiments, the plant glycogenin-like nucleic acid molecules and polypeptides do not include sequences consisting of those sequences known in the art. For example, in one embodiment, the plant glycogenin-like nucleic acid molecules do not include EST sequences.

In other embodiments, the plant glycogenin-like nucleic acid molecules of the invention, encode polypeptides that function as plant glycogenin-like proteins. The functionality of such nucleic acid molecules can be assessed using the yeast hybrid complementation assay as described herein in Example 3. Alternatively, the functionality of such nucleic acid molecules can be assessed using a complementation assay in *Arabidopsis* as described in this section.

An isolated nucleic acid molecule encoding a variant protein can be created by introducing one or more nucleotide substitutions, additions or deletions into the plant glycogenin-like nucleic acid molecule, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as, ethyl methane sulfonate, X-rays, gamma rays, T-DNA mutagenesis, or site-directed mutagenesis, PCR-mediated mutagenesis. Briefly, PCR primers

are designed that delete the trinucleotide codon of the amino acid to be changed and replace it with the trinucleotide codon of the amino acid to be included. This primer is used in the PCR amplification of DNA encoding the protein of interest. This fragment is then isolated and inserted into the full length cDNA encoding the protein of interest and expressed recombinantly.

An isolated nucleic acid molecule encoding a variant protein can be created by any of the methods described in section 1.1. Either conservative or non-conservative amino acid substitutions can be made at one or more amino acid residues. Both conservative and non-conservative substitutions can be made. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are can be divided into four families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) nonpolar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. In similar fashion, the amino acid repertoire can be grouped as (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine histidine, (3) aliphatic = glycine, alanine, valine, leucine, isoleucine, serine, threonine, with serine and threonine optionally be grouped separately as aliphatic-hydroxyl; (4) aromatic = phenylalanine, tyrosine, tryptophan; (5) amide = asparagine, glutamine; and (6) sulfur - containing = cysteine and methionine. (*See*, for example, Biochemistry, 4th ed., Ed. by L. Stryer, WH Freeman and Co.: 1995).

Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

The invention also encompasses (a) DNA vectors that contain any of the foregoing nucleic acids and/or coding sequences (i.e. fragments and variants) and/or their complements (*i.e.*, antisense molecules); (b) DNA expression vectors that contain any of the foregoing nucleic acids and/or coding sequences operatively associated with a regulatory region that directs the expression of the nucleic acids and/or coding sequences; and (c) genetically

engineered host cells that contain any of the foregoing nucleic acids and/or coding sequences operatively associated with a regulatory region that directs the expression of the gene and/or coding sequences in the host cell. As used herein, regulatory region include, but are not limited to, inducible and non-inducible genetic elements known to those skilled in the art that drive and regulate expression of a nucleic acid. The nucleic acid molecules of the invention may be under the control of a promoter, enhancer, operator, cis-acting sequences, or trans-acting factors, or other regulatory sequence. The nucleic acid molecules encoding regulatory regions of the invention may also be functional fragments of a promoter or enhancer. The nucleic acid molecules encoding a regulatory region is preferably one which will target expression to desired cells, tissues, or developmental stages.

Examples of highly suitable nucleic acid molecules encoding regulatory regions are endosperm specific promoters, such as that of the high molecular weight glutenin (HMWG) gene of wheat, prolamin, or ITR1, or other suitable promoters available to the skilled person such as gliadin, branching enzyme, ADFG pyrophosphorylase, patatin, starch synthase, rice actin, and actin, for example.

Other suitable promoters include the stem organ specific promoter gSPO-A, the seed specific promoters Napin, KTI 1, 2, & 3, beta-conglycinin, beta-phaseolin, heliathin, phytohemaglutinin, legumin, zein, lectin, leghemoglobin c3, ABI3, PvAlf, SH-EP, EP-C1, 2S1, EM 1, and ROM2.

Constitutive promoters, such as CaMV promoters, including CaMV 35S and CaMV 19S may also be suitable. Other examples of constitutive promoters include Actin 1, Ubiquitin 1, and HMG2.

In addition, the regulatory region of the invention may be one which is environmental factor-regulated such as promoters that respond to heat, cold, mechanical stress, light, ultra-violet light, drought, salt and pathogen attack. The regulatory region of the invention may also be one which is a hormone-regulated promoter that induces gene expression in response to phytohormones at different stages of plant growth. Useful inducible promoters include, but are not limited to, the promoters of ribulose bisphosphate carboxylase (RUBISCO) genes, chlorophyll a/b binding protein (CAB) genes, heat shock genes, the defense responsive gene (*e.g.*, phenylalanine ammonia lyase genes), wound induced genes (*e.g.*, hydroxyproline rich

cell wall protein genes), chemically-inducible genes (*e.g.*, nitrate reductase genes, gluconase genes, chitinase genes, PR-1 genes *etc.*), dark-inducible genes (*e.g.*, asparagine synthetase gene as described by U.S. Patent 5,256,558), and developmental-stage specific genes (*e.g.*, Shoot Meristemless gene, ABI3 promoter and the 2S1 and Em 1 promoters for seed development (Devic et al.,1996, Plant Journal 9(2):205-215), and the kin1 and cor6.6 promoters for seed development (Wang et al., 1995, Plant Molecular Biology, 28(4):619-634). Examples of other inducible promoters and developmental-stage specific promoters can be found in Datla et al., in particular in Table 1 of that publication (Datla et al., 1997, Biotechnology annual review 3:269-296).

A vector of the invention may also contain a sequence encoding a transit peptide which can be fused in-frame such that it is expressed as a fusion protein.

Methods which are well known to those skilled in the art can be used to construct vectors and/or expression vectors containing plant glycogenin-like protein coding sequences and appropriate transcriptional/translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, and Ausubel et al., 1989. Alternatively, RNA capable of encoding plant glycogenin-like protein sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in Gait, 1984, *Oligonucleotide Synthesis*, IRL Press, Oxford. In a preferred embodiment of the invention, the techniques described in Example 6, and illustrated in Figure 6 are used to construct a vector.

A variety of host-expression vector systems may be utilized to express the plant glycogenin-like gene products of the invention. Such host-expression systems represent vehicles by which the plant glycogenin-like gene products of interest may be produced and subsequently recovered and/or purified from the culture or plant (using purification methods well known to those skilled in the art), but also represent cells which may, when transformed or transfected with the appropriate nucleic acid molecules, exhibit the plant glycogenin-like protein of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing plant glycogenin-like protein coding

sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the plant glycogenin-like protein coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the plant glycogenin-like protein coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV); plant cell systems transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing plant glycogenin-like protein coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter; the cytomegalovirus promoter/enhancer; etc.). In a preferred embodiment of the invention, an expression vector comprising a plant glycogenin-like nucleic acid molecule operably linked to at least one suitable regulatory sequence is incorporated into a plant by one of the methods described in this section, section 1.3, 1.4 and 1.5 or in Examples 7, 8, 9, and 12.

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the plant glycogenin-like protein being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of antibodies or to screen peptide libraries, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the plant glycogenin-like coding sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-9; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 264:5503-9); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene protein can be released from the GST moiety.

In one such embodiment of a bacterial system, full length cDNA nucleic acid molecules are appended with in-frame Bam HI sites at the amino terminus and Eco RI sites at the carboxyl terminus using standard PCR methodologies (Innis et al., 1990, *supra*) and ligated into the pGEX-2TK vector (Pharmacia, Uppsala, Sweden). The resulting cDNA construct contains a kinase recognition site at the amino terminus for radioactive labeling and glutathione S-transferase sequences at the carboxyl terminus for affinity purification (Nilsson, et al., 1985, *EMBO J.* 4:1075; Zabeau and Stanley, 1982, *EMBO J.* 1: 1217).

The recombinant constructs of the present invention may include a selectable marker for propagation of the construct. For example, a construct to be propagated in bacteria preferably contains an antibiotic resistance gene, such as one that confers resistance to kanamycin, tetracycline, streptomycin, or chloramphenicol. Examples of other suitable marker genes include antibiotic resistance genes such as those conferring resistance to G4 18 and hygromycin (*npt-II, hyg*-B); herbicide resistance genes such as those conferring resistance to phosphinothricin and sulfonamide based herbicides (*bar* and *sul* respectively; EP-A-242246, EP-A- 0369637) and screenable markers such as beta-glucoronidase (GB2 197653), luciferase and green fluorescent protein. Suitable vectors for propagating the construct include, but are not limited to, plasmids, cosmids, bacteriophages or viruses.

The marker gene is preferably controlled by a second promoter which allows expression in cells other than the seed, thus allowing selection of cells or tissue containing the marker at any stage of development of the plant. Preferred second promoters are the promoter of nopaline synthase gene of *Agrobacterium* and the promoter derived from the gene which encodes the 35S subunit of cauliflower mosaic virus (CaMV) coat protein. However, any other suitable second promoter may be used.

The nucleic acid molecule encoding a plant glycogenin-like protein may be native or foreign to the plant into which it is introduced. One of the effects of introducing a nucleic acid molecule encoding a plant glycogenin-like gene into a plant is to increase the amount of plant glycogenin-like protein present and therefore the amount of starch produced by increasing the copy number of the nucleic acid molecule. Foreign plant glycogenin-like nucleic acid molecules may in addition have different temporal and/or spatial specificity for starch synthesis compared to the native plant glycogenin-like protein of the plant, and so may

be useful in altering when and where or what type of starch is produced. Regulatory elements of the plant glycogenin-like genes may also be used in altering starch synthesis in a plant, for example by replacing the native regulatory elements in the plant or providing additional control mechanisms. The regulatory regions of the invention may confer expression of a plant glycogenin-like gene product in a chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, tissue-specific, or constitutive manner. Alternatively, the expression conferred by a regulatory region may encompass more than one type of expression selected from the group consisting of chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, tissue-specific, and constitutive.

Further, any of the nucleic acid molecules (including EST clone nucleic acid molecules) and/or polypeptides and proteins described herein, can be used as markers for qualitative trait loci in breeding programs for crop plants. To this end, the nucleic acid molecules, including, but not limited to, full length plant glycogenin-like genes coding sequences, and/or partial sequences (ESTs), can be used in hybridization and/or DNA amplification assays to identify the endogenous plant glycogenin-like genes, plant glycogenin-like gene mutant alleles and/or plant glycogenin-like gene expression products in cultivars as compared to wild-type plants. They can also be used as markers for linkage analysis of qualitative trait loci. It is also possible that the plant glycogenin-like genes may encode a product responsible for a qualitative trait that is desirable in a crop breeding program. Alternatively, the plant glycogenin-like protein and/or peptides can be used as diagnostic reagents in immunoassays to detect expression of the plant glycogenin-like genes in cultivars and wild-type plants.

Genetically-engineered plants containing constructs comprising the plant glycogenin-like nucleic acid and a reporter gene can be generated using the methods described herein for each plant glycogenin-like nucleic acid gene variant, to screen for loss-of-function variants induced by mutations, including but not limited to, deletions, point mutations, rearrangements, translocation, etc. The constructs can encode for fusion proteins comprising a plant glycogenin-like protein fused to a protein product encoded by a reporter gene.

Alternatively, the constructs can encode for a plant glycogenin-like protein and a reporter gene product that are not fused. The constructs may be transformed into the homozygous recessive plant glycogenin-like gene mutant background, and the restorative phenotype examined, i.e. quantity and quality of starch, as a complementation test to confirm the functionality of the variants isolated.

1.1 PLANT GLYCOGENIN-LIKE GENE PRODUCTS

The invention encompasses the polypeptides of SEQ ID Nos: 3, 7, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 31, 32, or 34. Plant glycogenin-like proteins, polypeptides and peptide fragments, variants, allelic variants, mutated, truncated or deleted forms of plant glycogenin-like proteins and/or plant glycogenin-like fusion proteins can be prepared for a variety of uses, including, but not limited to, the generation of antibodies, as reagents in assays, the identification of other cellular gene products involved in starch synthesis and/or starch synthesis initiation, etc.

Plant glycogenin-like translational products include, but are not limited to those proteins and polypeptides encoded by the sequences of the plant glycogenin-like nucleic acid molecules of the invention. The invention encompasses proteins that are functionally equivalent to the plant glycogenin-like gene products of the invention.

The primary use of the plant glycogenin-like gene products of the invention is to alter starch synthesis via increasing the number of priming or initiation sites for elongation of glucose chains.

In an embodiment of the invention, an isolated polypeptide comprises the amino acid molecule of SEQ ID NO: 9 or a variant or fragment thereof, provided the polypeptide sequence is not that of SEQ ID NO: 35.

The present invention also provides variants of the polypeptides of the invention. Such variants have an altered amino acid sequence which can function as either agonists (mimetics) or as antagonists. Variants can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for

example, deleting one or more of the receiver domains. Thus, specific biological effects can be elicited by addition of a variant of limited function.

Modification of the structure of the subject polypeptides can be for such purposes as enhancing efficacy, stability, or post-translational modifications (*e.g.*, to alter the phosphorylation pattern of the protein). Such modified peptides, when designed to retain at least one activity of the naturally-occurring form of the protein, or to produce specific antagonists thereof, are considered functional equivalents of the polypeptides. Such modified peptides can be produced, for instance, by amino acid substitution, deletion, or addition.

For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*i.e.* isosteric and/or isoelectric mutations) will not have a major effect on the biological activity of the resulting molecule.

Whether a change in the amino acid sequence of a peptide results in a functional homolog (*e.g.*, functional in the sense that the resulting polypeptide mimics or antagonizes the wild-type form) can be readily determined by assessing the ability of the variant peptide to produce a response in cells in a fashion similar to the wild-type protein, or competitively inhibit such a response. Polypeptides in which more than one replacement has taken place can readily be tested in the same manner.

In a preferred embodiment, a mutant polypeptide that is a variant of a polypeptide of the invention can be assayed for: (1) the ability to complement glycogenin function in a yeast or plant system in which the native glycogenin or plant glygogenin-like genes have been knocked out; (2) the ability to form a complex with a glucose or oligosaccharide; or (3) the ability to promote initiation of elongation of polysaccharide chains.

The invention encompasses functionally equivalent mutant plant glycogenin-like proteins and polypeptides. The invention also encompasses mutant plant glycogenin-like proteins and polypeptides that are not functionally equivalent to the gene products. Such a mutant plant glycogenin-like protein or polypeptide may contain one or more deletions, additions or substitutions of plant glycogenin-like amino acid residues within the amino acid sequence encoded by any one the plant glycogenin-like nucleic acid molecules described

above in Section 1.1, and which result in loss of one or more functions of the plant glycogenin-like protein, thus producing a plant glycogenin-like gene product not functionally equivalent to the wild-type plant glycogenin-like protein.

Plant glycogenin-like proteins and polypeptides bearing mutations can be made to plant glycogenin-like DNA (using techniques discussed above as well as those well known to one of skill in the art) and the resulting mutant plant glycogenin-like proteins tested for activity. Mutants can be isolated that display increased function, (*e.g.*, resulting in improved root formation), or decreased function (*e.g.*, resulting in suboptimal root function). In particular, mutated plant glycogenin-like proteins in which any of the exons shown in SEQ ID NO: 1 are deleted or mutated are within the scope of the invention. Additionally, peptides corresponding to one or more exons of the plant glycogenin-like protein, truncated or deleted plant glycogenin-like protein are also within the scope of the invention. Fusion proteins in which the full length plant glycogenin-like protein or a plant glycogenin-like polypeptide or peptide fused to an unrelated protein are also within the scope of the invention and can be designed on the basis of the plant glycogenin-like nucleotide and plant glycogenin-like amino acid sequences disclosed herein.

While the plant glycogenin-like polypeptides and peptides can be chemically synthesized (*e.g.*, see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., NY) large polypeptides derived from plant glycogenin-like gene and the full length plant glycogenin-like gene may advantageously be produced by recombinant DNA technology using techniques well known to those skilled in the art for expressing nucleic acid molecules.

Nucleotides encoding fusion proteins may include, but are not limited to, nucleotides encoding full length plant glycogenin-like proteins, truncated plant glycogenin-like proteins, or peptide fragments of plant glycogenin-like proteins fused to an unrelated protein or peptide, such as for example, an enzyme, fluorescent protein, or luminescent protein that can be used as a marker or an epitope that facilitates affinity-based purificaiton. Alternatively, the fusion protein can further comprise a heterologous protein such as a transit peptide or fluorescence protein.

In an embodiment of the invention, the percent identity between two polypeptides of

the invention is at least 40%. In a preferred embodiment of the invention, the percent identity between two polypeptides of the invention is at least 50%. In another embodiment, the percent the percent identity between two polypeptides of the invention is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, or at least 98%. Determining whether two sequences are substantially similar may be carried out using any methodologies known to one skilled in the art, preferably using computer assisted analysis as described in section 1.1.

Further, it may be desirable to include additional DNA sequences in the protein expression constructs. Examples of additional DNA sequences include, but are not limited to, those encoding: a 3' untranslated region; a transcription termination and polyadenylation signal; an intron; a signal peptide (which facilitates the secretion of the protein); or a transit peptide (which targets the protein to a particular cellular compartment such as the nucleus, chloroplast, mitochondria or vacuole). The nucleic acid molecules of the invention will preferably comprise a nucleic acid molecule encoding a transit peptide, to ensure delivery of any expressed protein to the plastid. Preferably the transit peptide will be selective for plastids such as amyloplasts or chloroplasts, and can be native to the nucleic acid molecule of the invention or derived from known plastid sequences, such as those from the small subunit of the ribulose bisphosphate carboxylase enzyme (ssu of rubisco) from pea, maize or sunflower for example. Transit peptide comprising amino acid residues 1-65 of SEQ ID NO: 2 is an example of a transit peptide native to the polypeptide of the invention. Where an agonist or antagonist which modulates activity of the plant glycogenin-like protein is a polypeptide, the polypeptide itself must be appropriately targeted to the plastids, for example by the presence of plastid targeting signal at the N terminal end of the protein (Castro Silva Filho *et al* Plant Mol Biol 30 769-780 (1996) or by protein-protein interaction (Schenke PC *et al*, Plant Physiol 122 235-241 (2000) and Schenke *et al* PNAS 98(2) 765-770 (2001). The transit peptides of the invention are used to target transportation of plant glycogenin-like proteins as well as agonists or antagonists thereof to plastids, the sites of starch synthesis, thus altering the starch synthesis process and resulting starch characteristics.

The plant glycogenin-like proteins and transit peptides associated with the plant glycogenin-like genes of the present invention have a number of important agricultural uses. The transit peptides associated with the plant glycogenin-like genes of the invention may be

used, for example, in transportation of desired heterologous gene products to a root, a root modified through evolution, tuber, stem, a stem modified through evolution, seed, and/or endosperm of transgenic plants transformed with such constructs.

The invention encompasses methods of screening for agents (i.e., proteins, small molecules, peptides) capable of altering the activity of a plant glycogenin-like protein in a plant. Variants of a protein of the invention which function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the protein of the invention for agonist or antagonist activity. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into nucleic acid molecules such that a degenerate set of potential protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display). There are a variety of methods which can be used to produce libraries of potential variants of the polypeptides of the invention from a degenerate oligonucleotide sequence. Methods for synthesizing degenerate oligonucleotides are known in the art (*see, e.g.*, Narang, 1983, *Tetrahedron* 39:3; Itakura et al., 1984, *Annu. Rev. Biochem.* 53:323; Itakura et al., 1984, *Science* 198:1056; Ike et al., 1983, *Nucleic Acid Res.*11:477).

In addition, libraries of fragments of the coding sequence of a polypeptide of the invention can be used to generate a variegated population of polypeptides for screening and subsequent selection of variants. For example, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of the coding sequence of interest with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the protein of interest.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify variants of a protein of the invention (Arkin and Yourvan, 1992, *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al., 1993, *Protein Engineering* 6(3):327-331).

An isolated polypeptide of the invention, or a fragment thereof, can be used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. The full-length polypeptide or protein can be used or, alternatively, the invention provides antigenic peptide fragments for use as immunogens. In one embodiment, the antigenic peptide of a protein of the invention or fragments or immunogenic fragments of a protein of the invention comprise at least 8 (preferably 10, 15, 20, 30 or 35) consecutive amino acid residues of the amino acid sequence of SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, or 34 and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

Exemplary amino acid sequences of the polypeptides of the invention can be used to generate antibodies against plant glycogenin-like genes. In one embodiment, the immunogenic polypeptide is conjugated to keyhole limpet hemocyanin ("KLH") and injected into rabbits. Rabbit IgG polyclonal antibodies can purified, for example, on a peptide affinity column. The antibodies can them be used to bind to and identify the polypeptides of the invention that have been extracted and separated via gel electrophoresis or other means.

One aspect of the invention pertains to isolated plant glycogenin-like polypeptides of the invention, variants thereof, as well as variants suitable for use as immunogens to raise antibodies directed against a plant glycogenin-like polypeptide of the invention. In one

embodiment, the native polypeptide can be isolated, using standard protein purification techniques, from cells or tissues expressing a plant glycogenin-like polypeptide. In a preferred embodiment, plant glycogenin-like polypeptides of the invention are produced from expression vectors by recombinant DNA techniques. In another preferred embodiment, a polypeptide of the invention is synthesized chemically using standard peptide synthesis techniques.

An isolated or purified protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free" indicates protein preparations in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, protein that is substantially free of cellular material includes protein preparations having less than 20%, 10%, or 5% (by dry weight) of a contaminating protein. Similarly, when an isolated plant glycogenin-like polypeptide of the invention is recombinantly produced, it is substantially free of culture medium. When the plant glycogenin-like polypeptide is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals.

Biologically active portions of a polypeptide of the invention include polypeptides comprising amino acid sequences identical to or derived from the amino acid sequence of the protein, such that the variants sequences comprise conservative substitutions or truncations (*e.g.*, amino acid sequences comprising fewer amino acids than those shown in any of SEQ ID NOs: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, and 34, but which maintain a high degree of homology to the remaining amino acid sequence). Typically, biologically active portions comprise a domain or motif with at least one activity of the corresponding protein. Domains or motifs include, but are not limited to, a biologically active portion of a protein of the invention can be a polypeptide which is, for example, at least 10, 25, 50, 100, 200, 300, 400 or 500 amino acids in length. Polypeptides of the invention can comprise, for example, a glycosylation domain or site for complexing with polysaccharide or for attachment of disaccharide or a monomeric unit thereof, or a site that interacts with starch synthase and other enzymes that act on the polysaccharide.

1.2 PRODUCTION OF TRANSGENIC PLANTS AND PLANT CELLS

The invention also encompasses transgenic or genetically-engineered plants, and progeny thereof. As used herein, a transgenic or genetically-engineered plant referes to a plant and a portion of its progeny which comprises a nucleic acid molecule which is not native to the initial parent plant. The introduced nucleic acid molecule may originate from the same species e.g., if the desired result is over-expression of the endogenous gene, or from a different species. A transgenic or genetically-engineered plant may be easily identified by a person skilled in the art by comparing the genetic material from a non-transformed plant, and a plant produced by a method of the present invention for example, a transgenic plant may comprise multiple copies of plant glycogenin-like genes, and/or foreign nucleic acid molecules. Transgenic plants are readily distinguishable from non-transgenic plants by standard techniques. For example a PCR test may be used to demonstrate the presence or absence of introduced genetic material. Transgenic plants may also be distinguished from non-transgenic plants at the DNA level by Southern blot or at the RNA level by Northern blot or at the protein level by western blot, by measurement of enzyme activity or by starch composition or properties.

The nucleic acids of the invention may be introduced into a cell by any suitable means. Preferred means include use of a disarmed Ti-plasmid vector carried by Agrobacterium by procedures known in the art, for example as described in EP-A-01 16718 and EP-A-0270822. Agrobacterium mediated transformation methods are now available for monocots, for example as described in EP 0672752 and WO00/63398. Alternatively, the nucleic acid may be introduced directly into plant cells using a particle gun. A further method would be to transform a plant protoplast, which involves first removing the cell wall and introducing the nucleic acid molecule and then reforming the cell wall. The transformed cell can then be grown into a plant.

In an embodiment of the present invention, *Agrobacterium* is employed to introduce the gene constructs into plants. Such transformations preferably use binary *Agrobacterium* T-DNA vectors (Bevan, 1984, *Nuc. Acid Res.* 12:8711-21), and the co-cultivation procedure (Horsch et al., 1985, *Science* 227:1229-31). Generally, the *Agrobacterium* transformation

system is used to engineer dicotyledonous plants (Bevan et al., 1982, *Ann. Rev. Genet.* 16:357-84; Rogers et al., 1986, *Methods Enzymol.* 118:627-41). The *Agrobacterium* transformation system may also be used to transform, as well as transfer, DNA to monocotyledonous plants and plant cells (*see* Hernalsteen et al., 1984, *EMBO J.* 3:3039-41; Hooykass-Van Slogteren et al., 1984, *Nature* 311:763-4; Grimsley et al., 1987, *Nature* 325:1677-79; Boulton et al., 1989, *Plant Mol. Biol.* 12:31-40.; Gould et al., 1991, *Plant Physiol.* 95:426-34).

Various alternative methods for introducing recombinant nucleic acid constructs into plants and plant cells may also be utilized. These other methods are particularly useful where the target is a monocotyledonous plant or plant cell. Alternative gene transfer and transformation methods include, but are not limited to, protoplast transformation through calcium-, polyethylene glycol (PEG)- or electroporation-mediated uptake of naked DNA (*see* Paszkowski et al., 1984, *EMBO J.* 3:2717-22; Potrykus et al., 1985, *Mol. Gen. Genet.* 199:169-177; Fromm et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:5824-8; Shimamoto, 1989, *Nature* 338:274-6), and electroporation of plant tissues (D'Halluin et al., 1992, *Plant Cell* 4:1495-1505). Additional methods for plant cell transformation include microinjection, silicon carbide mediated DNA uptake (Kaeppler et al., 1990, *Plant Cell Reporter* 9:415-8), and microprojectile bombardment (Klein et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:4305-9; Gordon-Kamm et al., 1990, *Plant Cell* 2:603-18).

According to the present invention, desired plants and plant cells may be obtained by engineering the gene constructs described herein into a variety of plant cell types, including, but not limited to, protoplasts, tissue culture cells, tissue and organ explants, pollen, embryos as well as whole plants. In an embodiment of the present invention, the engineered plant material is selected or screened for transformants (*i.e.*, those that have incorporated or integrated the introduced gene construct or constructs) following the approaches and methods described below. An isolated transformant may then be regenerated into a plant. Alternatively, the engineered plant material may be regenerated into a plant, or plantlet, before subjecting the derived plant, or plantlet, to selection or screening for the marker gene traits. Procedures for regenerating plants from plant cells, tissues or organs, either before or after selecting or screening for marker gene or genes, are well known to those skilled in the

art.

A transformed plant cell, callus, tissue or plant may be identified and isolated by selecting or screening the engineered plant material for traits encoded by the marker genes present on the transforming DNA. For instance, selection may be performed by growing the engineered plant material on media containing inhibitory amounts of the antibiotic or herbicide to which the transforming marker gene construct confers resistance. Further, transformed plants and plant cells may also be identified by screening for the activities of any visible marker genes (*e.g.*, the ß-glucuronidase, luciferase, gréen fluorescent protein, B or C1 anythocyanin genes) that may be present on the recombinant nucleic acid constructs of the present invention. Such selection and screening methodologies are well known to those skilled in the art.

The present invention is applicable to all plants which produce or store starch. Examples of such plants are cereals such as maize, wheat, rice, sorghum, barley; fruit producing species such as banana, apple, tomato or pear; root crops such as cassava, potato, yam, beet or turnip; oilseed crops such as rapeseed, canola, sunflower, oil palm, coconut, linseed or groundnut; meal crops such as soya, bean or pea; and any other suitable species.

In a preferred embodiment of the present invention, the method comprises the additional step of growing the plant and harvesting the starch from a plant part. In order to harvest the starch, it is preferred that the plant is grown until plant parts containing starch develop, which may then be removed. In a further preferred embodiment, the propagating material from the plant may be removed, for example the seeds. The plant part can be an organ such as a stem, root, leaf, or reproductive body. Alternatively, the plant part may be a modified organ such as a tuber, or the plant part is a tissue such as endosperm.

1.3 TRANSGENIC PLANTS THAT ECTOPICALLY EXPRESS PLANT GLYCOGENIN-LIKE PROTEIN

According to one aspect of the invention, a nucleic acid molecule according to the invention is expressed in the plant cell, plant, or part of a plant that comprises a nucleotide sequence encoding a plant glycogenin-like protein, fragment of variant thereof. The nucleic

acid molecule expressed in the plant cell can comprise a nucleotide sequence encoding a full length plant glycogenin-like protein. Examples of such sequences include SEQ ID NOs: 1, 2, 6, 8, 10, 12, and 14, or variants thereof and the corresponding the amino acid sequences of SEQ ID NOs: 3, 7, 9, 11, 13, and 15 or variants thereof.

In an embodiment of the invention, the nucleic acid molecules of the invention are expressed in a plant cell and are transcribed only in the sense orientation. A plant that expresses a recombinant plant glycogenin-like nucleic acid may be engineered by transforming a plant cell with a nucleic acid construct comprising a regulatory region operably associated with a nucleic acid molecule, the sequence of which encodes a plant glycogenin-like protein or a fragment thereof. In plants derived from such cells, starch synthesis is altered in ways described in section 1.6. The term "operably associated" is used herein to mean that transcription controlled by the associated regulatory region would produce a functional mRNA, whose translation would produce the plant glycogenin-like protein. Starch may be altered in particular parts of a plant, including but not limited to seeds, tubers, leaves, roots and stems or modifications thereof.

In an embodiment of the invention, a plant is engineered to constitutively express a plant glycogenin-like protein in order to alter the starch content of the plant. In a preferred embodiment, the starch content is 40%, 30%, 20%, 10%, 5%, 2% greater than that of a non-engineered control plant(s). In another preferred embodiment, the starch content is 40%, 30%, 20%, 10%, 5%, 2% less than that of a non-engineered control plant(s).

In another aspect of the invention, where the nucleic acid molecules of the invention are expressed in a plant cell and are transcribed only in the sense orientation, the starch content of the plant cell and plants derived from such a cells exhibit altered starch content. The altered starch content comprises an increase in the ratio of amylose to amylopectin. In one embodiment of the invention, the ratio of amylose to amylopectin increases by 2%, 5%, 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In preferred embodiment of the invention, the nucleic acid molecules of the invention are expressed in a potato plant and are transcribed only in the sense orientation. The starch content of the plant, including the tubers, exhibit increased starch content. If the number of copies of the nucleic acid molecules of the invention are expressed in a potato plant that are

transcribed only in the sense orientation is increased, the starch content of the plant, including the tubers, increases.

In yet another embodiment of the present invention, it may be advantageous to transform a plant with a nucleic acid construct operably linking a modified or artificial promoter to a nucleic acid molecule having a sequence encoding a plant glycogenin-like protein or a fragment thereof. Such promoters typically have unique expression patterns and/or expression levels not found in natural promoters because they are constructed by recombining structural elements from different promoters. *See, e.g.*, Salina et al., 1992, *Plant Cell* 4:1485-93, for examples of artificial promoters constructed from combining cis-regulatory elements with a promoter core.

In a preferred embodiment of the present invention, the associated promoter is a strong root and/or embryo-specific plant promoter such that the plant glycogenin-like protein is overexpressed in the transgenic plant.

In yet another preferred embodiment of the present invention, the overexpression of plant glycogenin-like protein in starch producing organs and organelles may be engineered by increasing the copy number of the plant glycogenin-like gene. One approach to producing such transgenic plants is to transform with nucleic acid constructs that contain multiple copies of the complete plant glycogenin-like gene with native or heterolgous promoters. Another approach is repeatedly transform successive generations of a plant line with one or more copies of the complete plant glycogenin-like gene constructs. Yet another approach is to place a complete plant glycogenin-like gene in a nucleic acid construct containing an amplification-selectable marker (ASM) gene such as the glutamine synthetase or dihydrofolate reductase gene. Cells transformed with such constructs is subjected to culturing regimes that select cell lines with increased copies of complete plant glycogenin-like gene. *See, e.g.*, Donn et al., 1984, *J. Mol. Appl. Genet.* 2:549-62, for a selection protocol used to isolate of a plant cell line containing amplified copies of the GS gene. Cell lines with amplified copies of the plant glycogenin-like gene can then be regenerated into transgenic plants.

1.4 TRANSGENIC PLANTS THAT SUPPRESS ENDOGENOUS PLANT GLYCOGENIN-LIKE PROTEIN EXPRESSION

The nucleic acid molecules of the invention may also be used to augment the starch priming activity of a plant cell, plant, or part of a plant, or alternatively to alter activity of the plant glycogenin-like protein of a plant cell, plant, or part of a plant by modifying transcription or translation of the plant glycogenin-like gene. In an embodiment of the invention, an antagonist which is capable of altering the expression of a nucleic acid molecule of the invention is introduced into a plant in order to alter the synthesis of starch. The antagonist may be protein, nucleic acid, chemical antagonist, or any other suitable moiety. In an embodiment of the invention, an antagonist which is capable of altering the expression of a nucleic acid molecule of the invention is provided to alter the synthesis of starch. The antagonist may be protein, nucleic acid, chemical antagonist, or any other suitable moiety. Typically, the antagonist will function by inhibiting or enhancing transcription from the plant glycogenin-like gene, either by affecting regulation of the promoter or the transcription process; inhibiting or enhancing translation of any RNA product of the plant glycogenin-like gene; inhibiting or enhancing the activity of the plant glycogenin-like protein itself or inhibiting or enhancing the protein-protein interaction of the plant glycogenin-like protein and downstream enzymes of the starch biosynthesis pathway. For example, where the antagonist is a protein it may interfere with transcription factor binding to the plant glycogenin-like gene promoter, mimic the activity of a transcription factor, compete with or mimic the plant glycogenin-like protein, or interfere with translation of the plant glycogenin-like RNA, interfere with the interaction of the plant glycogenin-like protein and downstream enzymes. Antagonists which are nucleic acids may encode proteins described above, or may be transposons which interfere with expression of the plant glycogenin-like gene.

The suppression may be engineered by transforming a plant with a nucleic acid construct encoding an antisense RNA or ribozyme complementary to a segment or the whole of plant glycogenin-like gene RNA transcript, including the mature target mRNA. In another embodiment, plant glycogenin-like gene suppression may be engineered by transforming a plant cell with a nucleic acid construct encoding a ribozyme that cleaves the plant

glycogenin-like gene mRNA transcript.

In another embodiment, the plant glycogenin-like mRNA transcript can be suppressed through the use of RNA interference, referred to herein as RNAi. RNAi allows for selective knock out of a target gene in a highly effective and specific manner. The RNAi technique involves introducing into a cell double-stranded RNA (dsRNA) which corresponds to exon portions of a target gene such as an endogenous plant glycogenin-like gene. The dsRNA causes the rapid destruction of the target gene's messenger RNA, i.e. an endogenous plant glycogenin-like gene mRNA, thus preventing the production of the plant glycogenin-like protein encoded by that gene. The RNAi constructs of the invention confer expression of dsRNA which correspond to exon portions of an endogenous plant glycogenin-like gene. The strands of RNA that form the dsRNA are complimentary strands from encoded by coding region, i.e., exons encoding sequence, on the 3' end of the plant glycogenin-like gene.

The dsRNA has an effect on the stability of the mRNA. The mechanism of how dsRNA results in the loss of the targeted homologous mRNA is still not well understood (Cogoni and Macino, 2000, Genes Dev 10: 638-643; Guru, 2000, Nature 404, 804-808; Hammond et al., 2001, Nature Rev Gen 2: 110-119). Current theories suggest a catalytic or amplification process occurs that involves initiation step and an effector step.

In the initiation step, input dsRNA is digested into 21-23 nucleotide "guide RNAs". These guide RNAs are also referred to as siRNAs, or short interfering RNAs. Evidence indicates that siRNAs are produced when a nuclease complex, which recognizes the 3' ends of dsRNA, cleaves dsRNA (introduced directly or via a transgene or virus) ~22 nucleotides from the 3' end. Successive cleavage events, either by one complex or several complexes, degrade the RNA to 19-20 bp duplexes (siRNAs), each with 2-nucleotide 3' overhangs. RNase III-type endonucleases cleave dsRNA to produce dsRNA fragments with 2-nucleotide 3' tails, thus an RNase III-like activity appears to be involved in the RNAi mechanism. Because of the potency of RNAi in some organisms, it has been proposed that siRNAs are replicated by an RNA-dependent RNA polymerase (Hammond et al., 2001, Nature Rev Gen 2:110-119; Sharp, 2001, Genes Dev 15: 485-490).

In the effector step, the siRNA duplexes bind to a nuclease complex to form what is known as the RNA-induced silencing complex, or RISC. The nuclease complex responsible

for digestion of mRNA may be identical to the nuclease activity that processes input dsRNA to siRNAs, although its identity is currently unclear. In either case, the RISC targets the homologous transcript by base pairing interactions between one of the siRNA strands and the endogenous mRNA. It then cleaves the mRNA ~12 nucleotides from the 3' terminus of the siRNA (Hammond et al., 2001, Nature Rev Gen 2:110-119; Sharp, 2001, Genes Dev 15: 485-490).

Methods and procedures for successful use of RNAi technology in post-transcriptional gene silencing in plant systems has been described by Waterhouse et al. (Waterhouse et al., 1998, Proc Natl Acad Sci U S A, 95(23):13959-64). Methods specific to construction of the RNAi constructs of the invention can be found in Examples 2 and 6 as well as Figures 6 and 10. While the invention encompasses use of any plant glycogenin-like gene of the invention in the RNAi constructs, in a preferred embodiment, the strands of RNA that form the dsRNA are complimentary strands encoded by a coding region on the 3' end from nucleotide residues 1196-1662 of SEQ ID NO:2.

For all of the aforementioned suppression or antisense constructs, it is preferred that such nucleic acid constructs express specifically in organs where starch synthesis occurs (i.e. tubers, seeds, stems roots and leaves) and/or the plastids where starch synthesis occurs. Alternatively, it may be preferred to have the suppression or antisense constructs expressed constitutively. Thus, constitutive promoters, such as the nopaline, CaMV 35S promoter, may also be used to express the suppression constructs. A most preferred promoter for these suppression or antisense constructs is a rice actin promoter. Alternatively, a co-suppression construct promoter can be one that expresses with the same tissue and developmental specificity as the plant glycogenin-like gene.

In accordance with the present invention, desired plants with suppressed target gene expression may also be engineered by transforming a plant cell with a co-suppression construct. A co-suppression construct comprises a functional promoter operatively associated with a complete or partial plant glycogenin-like nucleic acid molecule. According to the present invention, it is preferred that the co-suppression construct encodes fully functional plant glycogenin-like gene mRNA or enzyme, although a construct encoding a an incomplete plant glycogenin-like gene mRNA may also be useful in effecting co-suppression.

In accordance with the present invention, desired plants with suppressed target gene expression may also be engineered by transforming a plant cell with a construct that can effect site-directed mutagenesis of the plant glycogenin-like gene. For discussions of nucleic acid constructs for effecting site-directed mutagenesis of target genes in plants see, *e.g.*, Mengiste et al., 1999, Biol. Chem. 380:749-758; Offringa et al., 1990, *EMBO J.* 9:3077-84; and Kanevskii et al., 1990, *Dokl. Akad. Nauk. SSSR* 312:1505-7. It is preferred that such constructs effect suppression of plant glycogenin-like genes by replacing the endogenous plant glycogenin-like gene nucleic acid molecule through homologous recombination with either an inactive or deleted plant glycogenin-like protein coding nucleic acid molecule.

In yet another embodiment, antisense technology can be used to inhibit plant glycogenin-like gene mRNA expression. Alternatively, the plant can be engineered, *e.g.*, via targeted homologous recombination to inactive or "knock-out" expression of the plant's endogenous plant glycogenin-like protein. The plant can be engineered to express an antagonist that hybridizes to one or more regulatory elements of the gene to interfere with control of the gene, such as binding of transcription factors, or disrupting protein-protein interaction. The plant can also be engineered to express a co-suppression construct. The suppression technology may also be useful in down-regulating the native plant glycogenin-like gene of a plant where a foreign plant glycogenin-like gene has been introduced. To be effective in altering the activity of a plant glycogenin-like protein in a plant, it is preferred that the nucleic acid molecules are at least 50, preferably at least 100 and more preferably at least 150 nucleotides in length. In one aspect of the invention, the nucleic acid molecule expressed in the plant cell can comprise a nucleotide sequence of the invention which encodes a full length plant glycogenin-like protein and wherein the nucleic acid molecule has been transcribed only in the antisense direction.

In a particular embodiment of the invention, a plant is engineered to express a dsRNA homologous to a portion of the coding region of an endogeneous PGSIP or a plant glycogenin-like gene transcribed in the antisense direction in order to alter the starch content of the plant. In a preferred embodiment, the starch content is 40%, 30%, 20%, 10%, 5% less than that of a non-engineered control plant(s). In a another preferred embodiment, starch is absent from certain plant organs or tissues in comparison to a non-engineered control

plant(s). In one embodiment starch content is decreased or absent in the leaves of plants engineered using the antisense technology described herein when compared to the starch content in a non-engineered control plant(s). In other embodiments the starch content of tubers, or seeds is decreased or absent in plants engineered using the antisense technology described herein when compared to the starch content in a non-engineered control plant(s). Plant tissues in which starch content can be decreased using the methods of the invention include but are not limited to endosperm, leaf mesophyll, and root or stem cortex or pith.

In another aspect of the invention, the nucleic acid molecules of the invention are expressed in a plant cell engineered expressing a dsRNA homologous to a portion of the coding region of an endogeneous PGSIP or using the antisense technology described herein and the starch content of the plant cell and plants derived from such a cells exhibit altered starch content. The altered starch content comprises an decrease in the ratio of amylose to amylopectin. In one embodiment of the invention, the ratio of amylose to amylopectin decreases by 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In a particular embodiment, the nucleic acid molecules of the invention are expressing a dsRNA homologous to a portion of the coding region of an endogeneous PGSIP or using the antisense technology described herein, in conjunction with a developmental specific promoter directed towards later stages of development. In this particular embodiment, starch content in leaves of a plant can decrease, while starch content in other organs and tissues of a plant are altered in the same or different ways.

In another particular embodiment, the nucleic acid molecules of the invention are expressing a dsRNA homologous to a portion of the coding region of an endogeneous PGSIP or using the antisense technology described herein in conjunction with a developmental specific promoter directed towards later stages of seed development, in cereals crops. In this embodiment, the ratio of small starch granules to large starch granules increases. An increased ratio of small to large starch granules results in greater accessibility of starch granules, which has certain industrial and commercial advantages related to extraction and processing of starch.

The progeny of the transgenic or genetically-engineered plants of the invention

containing the nucleic acids of the invention are also encompassed by the invention.

1.5 MODIFIED STARCH

The invention encompasses methods of altering starch synthesis in a plant and the resulting modified starch produced.

In the context of the present invention, "altering starch synthesis" means altering any aspect of starch production in the plant, from initiation by the starch primer to downstream aspects of starch production such as elongation, branching and storage, such that it differs from starch synthesis in the native plant. In the invention, this is achieved by altering the activity of the starch primer, which includes, but is not limited to, its function in initiating starch synthesis, its temporal and spatial distribution and specificity, and its interaction with downstream factors in the synthesis pathway. The effects of altering the activity of the starch primer may include, for example, increasing or decreasing the starch yield of the plant; increasing or decreasing the rate of starch production; altering temporal or spatial aspects of starch production in the plant; altering the initiation sites of starch synthesis; changing the optimum conditions for starch production; and altering the type of starch produced, for example in terms of the ratio of its different components. For example, the endosperm of mature wheat and barley grains contain two major classes of starch granules: large, early formed "A" granules and small, later formed "B" granules. Type A starch granules in wheat are about 20 μm diameter and type B around 5 μm in diameter (Tester, 1997, in : Starch Structure and Functionality, Frazier et al., eds., Royal Society of Chemistry, Cambridge, UK). Rice starch granules are typically less than 5 μm in diameter, while potato starch granules can be greater than 80 μm in diameter. The quality of starch in wheat and barley is greatly influenced by the ratio of A-granules to B-granules. Altering the activity of the starch primer will influence the number of granule initiation sites, which will be an important factor in determining the number and size of formed starch granules. The degree to which the starch priming activity of the plant is affected will depend at least upon the nature and of the nucleic acid molecule or antagonist introduced into the plant, and the amount present. By altering these variables, a person skilled in the art can regulate the degree to which starch synthesis is altered according to the desired end result.

The methods of the invention (i.e. engineering-a plant to express a construct comprising a plant glycogenin-like nucleic acid) can, in addition to altering the total quantity of starch, alter the fine structure of starch in several ways including but not limited to, altering the ratio of amylose to amylopectin, altering the length of amylose chains, altering the length of chains of amylopectin fractions of low molecular weight or high molecular weight fractions, or altering the ratio of low molecular weight or high molecular weight chains of amylopectin. The methods of the invention can also be utilized to alter the granule structure of starch, i.e. the ratio of large to small starch granules from a plant or a portion of a plant. The alteration in the structure of starch can in turn effect the functional characteristics of starch such as viscosity, elasticity, or rheological properties of the starch as measured using viscometric analysis. The modified starch can also be characterized by an alteration of more than one of the above- mentioned properties.

In an embodiment the length of amylose chains in starch extracted from a plant engineered express a construct comprising a plant glycogenin-like nucleic acid is decreased by at least 50, 100, 150, 200, 250, or 300 glucose units in length in comparison to amylose from non-modified starch from a plant of the same genetic background. In another embodiment, the length of amylose chains in starch is increased by at least 50, 100, 150, 200, 250, or 300 glucose units in length in comparison to amylose from non-modified starch from a plant of the same genetic background.

In an embodiment of the invention, the ratio of amylose to amylopectin decreases by 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In a preferred embodiment, the ratio of low molecular weight chains to high molecular weight chains of amylopectin is altered by 10%, 20%, 30%, 40%, or 50% in comparison to a non-engineered control plant(s).

In another preferred embodiment the average length of low molecular weight chains of amylopectin is altered by 5, 10, 15, 20, or 25 glucose units in length in comparison to a non-engineered control plant(s). In yet another preferred embodiment the average length of high molecular weight chains of amylopectin is altered by 10, 20, 30, 40, 50 , 60 , 70, or 80 glucose units in length in comparison to a non-engineered control plant(s).

According to one aspect of the invention, the ratio of small starch granules to large

granules is altered by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a non-engineered control plant(s).

In another aspect, the invention provides a complex comprising plant glycogenin-like proteins and plant polysaccharides. The inventors believe that members of the family of plant glycogenin-like proteins serve as primers for biosynthesis of a range of polysaccharides in plants, including but not limited to starch, hemicelluloses, and cellulose. The plant polysaccharides may be either homopolysaccharides comprising only a single type of monomeric unit or a heteropolysaccharides comprising two or more different kinds of monomeric units. Accordingly, it is contemplated that plant glycogenin-like proteins form complexes with such polysaccharides and its subunits. Glycosylated plant glycogenin-like proteins are encompassed in the invention. In the broadest sense, the invention encompasses a complex comprising a plant glycogenin-like protein and a number of monomeric units also referred to as subunits of the polysaccharides. Examples of monomeric units include but are not limited to glucose, xylose, mannose, galactose, ribose, and rhamnose, and may be a hexose, or a pentose, wherein the number ranges from a single to thousands of monomeric units, and wherein the linkages between the subunits may vary resulting in linear and/or branched structures. For example, starch and precursors of starch comprise of glucose subunits joined by either alpha 1, 4-glycosidic bonds or alpha 1, 6-glycosidic linkages; cellulose and precursors of cellulose comprise glucose subunits joined by beta 1, 4-glycosidic bonds. The number of monomeric units ranges from 1-3, 2-5, 4-10, 8-16, 15-30, 20-40, 30-60, 50-100, 75-200, 100-500, or 300-800 monomeric units. Alternatively, the number of monomeric units ranges from 1000-5000, 5000-10,000, or 10,000-15,000 monomeric units. Preferably, the polysaccharide or its precursor is attached to a hydroxyl group of a tyrosine residue of the plant glycogenin-like protein. Without being bound by any theory or any mechanism, during biosynthesis, additional subunits, either singly or as oligosaccharides are added to the complex such that the total number of subunits increase over a period of time.

In one embodiment, the invention encompasses complexes comprising plant glycogenin-like protein and starch. In a specific embodiment, the complexes of plant glycogenin-like protein and starch are purified. The starch molecule or its precursor including a single glucose subunit, can be attached to a hydroxyl group of a tyrosine residue

of the plant glycogenin-like protein. In various embodiments, in a population of complexes, the starch molecules that are complexed with the plant glycogenin-like proteins have different chain lengths and branching structures, for example, 1-3, 2-5, 4-10, 8-16, 15-30, 20-40, 30-60, 50-100, 75-200, 100-500, 200-700 glucose subunits. The polysaccharide complexed with the plant glycogenin-like proteins may consists of 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, or 190 glucose subunits in length. In preferred embodiments of the invention, the polysaccharide is amylopectin, amylose, or a combination of both.

The complexes of the invention can be used to identify sites of starch synthesis in stages of plant development. Briefly, the glycogenin-like protein can be labeled by means described herein and the complexes from tissues, cells, or organs can then be separated by size and compared among different stages of development.

The embodiments described in each section above apply to the other aspects of the invention, mutatis mutandis.

EXAMPLES

EXAMPLE 1: Identification of Plant Glycogenin-like Gene Homologues in *Arabidopsis*

Arabidopsis nucleic acid molecules showing similarities to yeast glycogenin genes were identified by sequence analysis. The sequence analysis programs used in the following examples are from the Wisconsin Package of computer programs (Deveraux et al., *Nucl. Acids Res.* 12: 387 (1984); available from Genetics Computer Group, Madison, WI). ESTs and genes were identified using the program BLAST (Basic Local Alignment Search Tool; Altschul, S.F. et al (1990) J. Mol. Biol. 215:403-410, see also www.ncbi.nlm.nih.gov/BLAST/).

The sequence comparison and identification program tblastx was used with the yeast glycogenin 1 (Glg1) gene (GenBank:U25546, Swiss_Prot (SP):P36143) to search against the *Arabidopsis* sequences collected in an in-house database comprising published plant

sequences. A number of hits to this gene were obtained. One of the hits was identified as EMBL:AC004260 version GI:2957150 which was annotated as "Sequencing in progress." Therefore, the region showing homology to the yeast Glg1 gene was extracted and a protein sequence was predicted using GENSCAN (a protein prediction program, Burge, C. and Karlin, S. (1997), J.Mol.Biol., http://genes.mit.edu/GENSCANinfo.html). A blastp analysis using this protein showed strong homology to the glycogenin genes from *C.elegans* (8e-22), human (2e-19) and yeast (8e-06). A search in the database at NCBI at a later date showed that this gene is listed as T14N5.1 with the accession number EMBL:AC004260 (SPTREMBL:O80649) and annotated as "Unknown protein". The protein sequence is set forth in SEQ ID NO: 6.

The in-house database described above was also searched with the yeast Glg2 gene (GB:U25436, SP:P47011) and the sequence identified above (accession EMBL:AC004260) using the program tblastn and tblastx. A number of further hits were identified. Out of the list of best hits, accession no. EMBL:AB026654, gene_id:MVE11.2 (SPTREMBL:Q9LSB1), showed strong homology to the glycogenin genes from *C.elegans* (1e-21), GYG2 human (3e-21) and yeast (5e-06). The genomic sequence representing this gene was extracted and is shown in SEQ ID NO: 1. Further analysis by the organelle prediction programs PREDOTAR and/or TargetP (Emanuelsson *et al.*, J. Mol. Biol. 300: 1005-1016 (2000)) showed that the protein comprises a transit peptide as shown in Table 1 below.

Table 1. TargetP V1.0 Prediction Results.

Number of input sequences: 1

Cleavage site predictions included.

Using PLANT networks.

Name	Length	cTP	mTP	SP	Other	Loc.	RC	TPlen
AT3g18660 cDNA	659	0.792	0.181	0.004	0.172	C	2	65

Performing blastp analysis using this protein against yeast sequences in an in-house database clearly showed sequence similarities to the yeast Glg1 and Glg2 gene. were and a

CD-ROM containing the full genome sequence of *Arabidopsis* was made available. A search of the *Arabidopsis* genome sequencing project database published (Nature 408: 791, (2000)) showed that EMBL:AB026654 corresponded to the sequence having accession no. AT3g18660. However AT3g18660 is reported to encode a protein of 575 amino acids whereas our analysis shows that this gene actually encodes a protein of 659 amino acids. A blastp analysis against the in-house database showed strong hits to five genes, EMBL:AC004260, AC000106, AC069144, AL035678 and AL035678 (corresponding to MIPS:at1g77130, at1g08990, at1g54940, at4g33330 and at4g33340). The sequences of these five genes are shown in SEQ ID NOs: 6, 8, 10, 12 and 14. The different accession numbers of these genes and their description in various databases are presented in Table 2.

Table 2:

Accession numbers of the genes in various databases:

MIPS	SPTREMBL	EMBL	GENE	Size
AT3g18660	Q9LSB1	AB026654	MVE11.2	659[a] aa
at1g77130	O80649	AC004260	T14N5.1	1201aa
at1g08990	O 04031	AC000106	F7g19.14	546[b]aa
at1g54940	Q 9FZ37	AC069144	F14C21.47	557aa
at4g33330	Q9SZB0	AL035678	F17M5.90	333aa
at4g33340	Q9SZB1	AL035678	F17M5.100	277aa

Note: [a]= The AT3g18660 gene sequence in the MATDB (MIPS) database is reported to encode a 575 aa protein. The analysis performed by the inventors indicates that (exon 2) of the AT3g18660 gene is missing in the MATDB (MIPS) database sequence and present in sequences of the AT3g18660 gene found in other databases.

[b] = The at1g08990 gene accession in the MATDB (MIPS) database is reported to encode a protein of 550 aa in MATDB (MIPS). The at1g08990 gene accession in other databases is 546aa in length.

Table 3: Comparison of AT3g18660 with other glycogenin-like genes from *Arabidopsis*:

	% identity nucleotide	% identity protein
AT3g18660 X at1g77130	68	65
AT3g18660 X at1g08990	61	50
AT3g18660 X at1g54940	61	49
AT3g18660 X at4g33330	60	58
AT3g18660 X at4g33340	60	46

Table 2 shows the percentage identity between AT3g18660 and other glycogenin genes from *Arabidopsis* using the programme BESTFIT of the GCG package. In each case, the full length nucleotide and peptide was compared to the AT3g18660 gene.

These levels of identity are consistent with the genes encoding proteins with the same function. For example, the two yeast glycogenin genes are about 50% identical to one another at the protein level and are both known to be involved in the same pathway; both are essential for the production of glycogen and one can complement for the function of the other.

It is interesting that the carboxyl terminal region of the protein encoded by at1g77130 shows homology to a starch synthase (dull1) from maize. In yeast, glycogenin and glycogen synthase physically interact. This finding may be the first indication that a similar scenario exists in plants. The at1g77130 gene appears to be a duplication of the AT3g18660 sequence, and the small region of homology with dull1 may indicate that during the course of evolution this gene has become physically close to dull1. Recently published work (Yanai et al ., 2001, Proc. Natl. Acad. Sci. USA 98(14): 7940-7945) suggests that a functional association between two genes can be derived from the existence of a fusion of the two as one continuous sequence in another genome. In yeast, it has been shown by experimentation that glycogenin and glycogen synthase physically interact and are associated together in an enzymatic complex to allow glycogen biosynthesis. The inventors believe that PGSIP interacts with soluble starch synthases at the start of the starch biosynthesis process. This could be the first

step in the formation of a biosynthetic starch enzymes complex where PGSIP acts as a template, starch synthases extend the chain followed by branching by starch branching enzymes and other starch synthesis enzymes. It is likely that biosynthesis starch enzymes become associated with the very first complex formed in the process of the synthesis of a starch polymer.

The sequences of the six genes listed in Table 2 were compared by BLAST against the *Arabidopsis* sequences in an in-house database and a further hit was obtained. The identified sequence corresponding to SPTREMBL: Q8W4AZ, EMBL: AY062695 encodes a protein of 618 amino acids that showed strong homology to the glycogenin genes (4e –26). Further analysis of the sequence indicated that the protein represents the C terminal domain of the At1g77130 gene (O80649, T14N5.1) and is also annotated as At1g77130, T14N5.1 which encodes an unknown protein. This sequence is set forth in SEQ ID NO: 23.

EXAMPLE 2: Isolation of cDNA Encoding *A. thaliana* Glycogenin Homologue

Primers were designed to clone a full length cDNA representing the accession number AB026654, gene_id:MVEl1.2 (at3g18660 (MIPS)) from an *Arabidopsis thaliana* cDNA pool. Sequencing the full length clone indicated that the gene encoded a protein of 659 amino-acids and consists of five exons. The cDNA sequence designated as SEQ ID NO: 2.

Arabidopsis thaliana was grown in growth cabinets with a 16 hours light and 8 hours dark period at a temperature of 22°C during the day and 17°C during the night. A mixed cDNA sample was made with total RNA from 10 different tissues mixed together in equal amounts: root, dividing cell culture, young leaf, mature leaf, stem, seedling, seed, flower buds + flowers, drought 6 days- and drought 10 days-subjected plants.

The primer used to make the first strand cDNA using Superscript II was from the original paper on PCR amplification by (Frohman *et al.* (1988) Proc. Natl. Acad. Sci. USA, 85:8998):

5 'GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT 3'.

1 µl of this cDNA was used to amplify the cDNA clone representing the accession number GTD:S:1870408 (gene id:MVE1 1.2) utilizing the primers G1gfl and G1g int1 and C1aF and

Glgstop2.

G1gfl primer: 5 '-GACCATGGCAAACTCTCCCGC-3'

G1g int1 primer: 5' -GCAGCATACTTTTCCAATTAC-3'

C1aF primer: 5'-GCAAGTTCCGGCTATGGCAGC-3'

Glgstop2 primer: 5 -GCGTCACAAGTTATGGCCGGG-3'

PCR conditions:

Five 50 µl reaction was set up as follows:

Composition	PCR Programme	
Water.....35.5µl	95°C	2 min (hot start)
10xbuffer.....5µl	95°C	3 min
4mMdNTPs.....2.5µl	55°C	30 sec
Pfu Turbo polymerase.....1µl	72°C	2 min:30 sec
4mM primers.....5µl	72°C	10 min (extension)
cDNA.....1µl		

Two products were obtained. These were cloned in pBluescript vector (SK-) (Stratagene) and a full length clone was obtained. The map of this plasmid is shown in Figure 1.

EXAMPLE 3: Functional Analysis of The *Arabidopsis* cDNA

Yeast contains two glycogenin genes Glgl (YKROS8w) and Glg2 (YJL137c). Double mutants in the above genes do not make any glycogen (Cheng *et al* (1995) Mol. and Cell Biology 15(12):6632-6640). Mutant yeast strains from the EUROSCARF (European

Saccharomyces Cerevisiae ARchives For Functional Analysis) collection were obtained from SRD GmbH, D61440, Germany along with the wild type. Single mutants in the Glgl and Glg2 genes were obtained in addition to the double mutant. Additionally a plasmid containing the entire Glg2 ORF including the promoter was also obtained. This plasmid was used as a positive control to establish a complementation assay. The description of the strains are:

Wild type

ORF	Accession no.	Strain	Genotype
	Y00000	BY4741	MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0

Single mutants:

ORF	Accession no.	Strain	Genotype
YKR058W	Y15129	G1G1 mutant	BY4742; Mat alpha; his3 Δ1; leu2Δ0; ura3Δ0; YKR058w::kanMX4
YJL137c	Y17003	g1g2 mutant	BY4742; Mat a; his3 Δ1; leu2Δ0; ura3Δ0; YJL137c::kanMX4

Double mutants:

Mutant Strains	Genotype
1. glgl/glg2 deleted	BY4742; Mat alpha; his3 Δ1; leu2Δ0; ura3Δ0; YKR058w::kanMX4; YJL137c::kanMX4

2. glgl/glg2 deleted	BY4742; Mat a; his3 Δ1; leu2Δ0; ura3Δ0; YKR058w::kanMX4; YJL137c::kanMX4

Plasmid

Plasmid name	Gene	Marker
PYCG_YJL137c(pRS416)	G1g2ORF+prometer	URA3

Glycogen defect assay

First, it was established that the wild type and the double mutants were indeed different. For this experiment, freshly grown wild type, and the double mutants were picked up from YPD plates and the cells were suspended in 100 µl of water in an eppendorf tube. To this tube approximately 100 µl of glass beads (Sigma) and 10-20 µl of undiluted Lugol solution (Sigma) was added. The cells were vortexed briefly, spun down for few seconds and assayed for color development. The wild type cells stained brown whereas the double mutants did not stain and appeared yellow.

Complementation assay

Double mutants were transformed with the plasmid pRS416 and the transformants were selected on CSM/Ura- plate (Uracil drop out plate). As a negative control, double mutants were transformed without the plasmid. Many colonies were obtained in the positive plate but no colonies were obtained from the negative control indicating that the transformation had worked. The transformed double mutants were grown overnight in CSM/Ura- liquid media along with wild type and single mutants. Next day OD_{600} was checked to ensure equal amounts of cells in each of the tubes. Approximately equal amounts of cells were taken in an eppendorf tube and to this equal amounts of glass bead were added followed by 10-20 µl of undiluted Lugol solution (Sigma). The cells were vortexed briefly and centrifuged for few seconds and assayed for colour development. Complementation was observed in the double mutants as they appeared blue similar to the single g1g1 and glg2 mutants.

Optimisation of the assay to distinguish wildtype and mutant strains

A small amount of the wildtype (WT) and glycogenin double mutant (Mut) yeast strains were picked up from a well-grown plate, resuspended in 1ml of water, and vortexed briefly. The cells were diluted further in 1ml of water and 50ul of the diluted cells were plated on YPD plates. The plate was incubated at 30°C for two days and afterwards the plates were exposed to iodine vapour by inverting the plates on top of a 500ml glass beaker containing iodine chips (Sigma) placed on a low heater under a fume cupboard briefly for 2-3 minutes. Afterwards the plates were left open in the fume cupboard briefly for 1 minute and the colour development was monitored. The WT cells stained brown and the double mutants (Mut) stained pale yellow.

Cloning PGSIP cDNA in into the pYES2 vector for complementation studies

Two constructs were made to do the experiment, one contained the full length PGSIP cDNA including the transit peptide (TP) and another in which the transit peptide was removed (No transit peptide : NTP), these were cloned into pYes2 vector (Invitrogen). Primers were designed to amplify the full length PGSIP cDNA with the transit peptide (primers TPF and TPR) and without the transit peptide (primers NTPF and NTPR) so that these could be cloned into the pYes2 vector. A BamHI restriction enzyme site was incorporated into the forward primers (TPF and NTPR) and a XhoI restriction enzyme site was incorporated into the reverse primers (TPR and NTPR). The NTP forward primer (NTPF) was designed in such a manner so that it annealed at nucleotide position 190 of the full length PGSIP sequence and an ATG initiation codon was inserted after the BamHI site to ensure that translation into protein could occur. This resulted in a cDNA sequence lacking the first 63 amino acids of the PGSIP cDNA sequence which represents the transit peptide as predicted by the Target P program (Emanuelsson et al, J. Mol. Biol. 300:1005-1016 (2000). The primer sequences were as follows:

TPF 5'-GGATCCGACCATGGCAAACTCTCCCGC-3'

TPR 5-CTCGAGGCGTCACAAGTTATGGCCGGG- 3'

NTPF 5'- GGATCCATGTGTTGTTGTTTCACCAAG-3'

NTPR 5'-CTCGAGGCGTCACAAGTTATGGCCGGG-3'

A 50 µl PCR reaction was set up with Pfu polymerase (Stratagene) as follows: a coocktail solution was made with 35.5µl water, 5µl 10X PCR buffer+, 2.5µl solution (20mM MgCl and 4mM dNTPs), 1µl Pfu polymerase, 5µl 4mM primers (TP/NTP), and 1µl cDNA (1/100dil). The PCR thermocycler program consisted of a 95°C 3min (hot start), followed by 30 cycles of 95°C for 30sec, 50°C for 30sec, and 72°C for 3min. The final step in the program held the temperature at 24°C.

The amplified fragment was run out on an agarose gel, cut out and purified using the 'Geneclean kit' according to the manufacturers instructions (Bio101). The purified cDNA fragments were ligated into pBluescript vector (Stratagene) cut with EcoRV resttriction enzyme. Positive clones were identified and these were sequenced. Clones with the correct sequences were then cut with the restriction enzymes BamHI and XhoI and ligated in pYes2 vector cut with the restriction enzymes BamHI and XhoI. Positive clones were identified and these were named, pTPYes (Figure 2) and pNTPYes (Figure 3). In these plasmids, the cDNA was under the control of the yeast Gal 1 promoter that is both glucose repressible and galactose inducible.

Complementation analysis with the Arabidopsis glycogenin gene

Yeast strains were transformed with the above plasmids following the method of Finley and Brent, 1995, (http://cmmg.biosci.wayne.edu/finlab/YTHprotocols.htm and links there in) in combination with the Clontech yeast transformation kit. From a freshly grown plate a 5ml culture of yeast strain (WT and Mut) was inoculated in YPD medium (Clontech) overnight with shaking at 30°C. Next day, 3ml freshly grown cells were inoculated into 150ml YPD medium, (OD600=0.2) and grown shaking at 30°C for 3-4 hours (OD600=0.7). 100ml cells were then transferred to two 50ml orange cap tubes and centrifuged at room temperature at 2000rpm for 3 minutes. The supernatant was discarded completely. The cells

were washed by resuspending them in 2.5ml of sterile water followed by centrifugation as before. The supernatant was discarded and the cells were resuspended by adding 625ul of Lithium Acetate (LiAc)/TE (10mM Tris HCL pH 7.5, 1mM EDTA, 100mM LiAc; made from a filter-sterile stock of 1M LiAc, pH 7.5) in each tube. The cells were centrifuged as before and the supernatant was discarded. The cells were resuspended in 250ml of LiAc/TE then pooled into a single eppendorf tube giving 500ml of competent yeast cells. In an eppendorf tube the following was prepared, 6ml Herring Testis DNA (Clontech,10mg/ml, boiled earlier for 10 minutes and quenched on ice), 8ml DNA [pYes2 empty plasmid, TPYes and NTP Yes DNA (~2ug)] and 6ml of water making a total volume of 20ml. In another tube 100ml of competent yeast cells were added to which the 20ml mixture made above, plus 11ml DMSO and 600ul of 40% PEG 4000 in LiAc/TE (made from stocks of 1M LiAc pH 7.5, filter sterile 50% PEG 4000 in water, 1M Tris HCl pH 7.5 and 0.5M EDTA) was added. The tubes were inverted three to four times gently and incubated at 30°C for 30 minutes. The tubes were inverted again gently and heat shocked at 42°C for 20minutes after which 50-100ml was directly plated on CSM/Ura-/glucose plates. The plates were incubated for two to three days at 30°C. Additionally, as a negative control, WT and Mut yeast strains were transformed with the empty pYes2 plasmid. As a positive control the Mut strains were transformed with the yeast GLG2 gene (plasmid pRS416) purchased from EUROSCARF. The transformed cells were selected on CSM/Ura- glucose drop out plates. After two days the cells were picked individually into patches and streaked onto glucose and galactose plates. In the end, we had the following plates.(Table 4)

Table 4

Name	*Glucose*	*Galactose*
1. WT:pYes2 control		*Yes*
2. Mut:pYes2 control	*Yes*	*Yes*
3. WT:NTP	*Yes*	*Yes*
4. Mut:NTP	*Yes*	*Yes*
5. WT:TP	*Yes*	*Yes*

6. Mut:TP	*Yes*	*Yes*
7. Mut:yeast GLG2 gene +ve control	*Yes*	*Yes*

Yeast strains used for the complementation experiment (Table 5)

Table 5

Name
1. WT:pYes2 control
2. Mut:pYes2 control
3. Mut:TP
4. Mut:NTP
5. Mut:yeastGLG2

The plates listed in Table 4 and Table 5 were grown for two days at 30°C as described above. The cells were diluted and plated on to both CSM/Ura- glucose and CSM/Ura-galactose plates. After two days of growth at 30°C the cells were exposed to iodine vapour as described above and photographs were taken. From the photographs, it was confirmed that the assay worked as the Mut strains containing the yeast GLG2 gene (no.7 from the table 4) stained brown both in the glucose and galactose plates. The WT strain (no.1 from the table 4) stained brown whereas the Mut strains (no. 2 from the table 4) containing the empty plasmid stained yellow. The cells containing the NTP plasmid (no. 4 from the table 4) stained yellow in glucose plate but it stained brown in galactose plates but the brown colour is not as intense as observed in Mut strains containing the yeast GLG2 gene indicating that the complementation is partial. This data indicates that the PGSIP cDNA is a functional orthologue of the yeast glycogenin gene and plays a role in starch biosynthesis especially in plants and particularly in *Arabidopsis*. The cells containing the TP plasmid (no. 3 from the table 4) stains yellow in glucose and galactose plates indicating that complementation was not achieved with this plasmid. In general, validating the function of plant genes by yeast complementation has been reported (Alderson et al, Proc. Natl. Acad.Sci. USA, 88:8602-

8605 (1991), Vogel et al., Plant J, 13 (5):673-683, 1998, Blazquez, et al., Plant J, 13 (5):685-689, 1998.

EXAMPLE 4: cDNA Isolation from Maize Endosperm

Maize EST identification

ESTs encoding corn glycogenin gene were identified using the program BLAST (Basic Local Alignment Search Tool; Altschul, S.F. *et al* (1990) J. Mol. Biol. 215:403-410, see also www.ncbi.nlm.nih.gov/BLAST/). A database search using the *Arabidopsis* gene AT3g18660 and atlg771 30 against the maize database at NCBI identified accession no. GB: BF729544 and GB: BG837930 which showed significant similarity to the Arabidopsis glycogenin genes. The sequence of the two ESTs is shown in SEQ ID NO: 4, and SEQ ID NO: 5 respectively. A blastx analysis of the two ESTs against SPTREMBL database showed that EST BF729544 picked up the first hit to the AT3g18660 gene whereas EST BG837930 showed first hit to the at1g77130 gene. Protein alignments of these ESTs indicated that both ESTs were partial and they showed 85-86% identity to the above two *Arabidopsis* genes. Moreover, for EST BF729544 the identity was confined to the central portion of the AT3g18669 protein starting at amino-acid position 245 and ending at position 427, whereas for EST BG837930 the identity started at amino-acid position 391 and extending until position 632. A bestfit analysis between the two nucleotide sequences of the ESTs and the AT3g18660 gene showed that the two ESTs have 68-69% identity. A bestfit analysis between the two EST DNA sequences showed that there was a high degree of homology between the two ESTs. From the above analysis, it appears that EST BF729544 is the homolog of the Arabidopsis AT3g18660 gene, whereas EST BG837930 is a homolog of the Arabidopsis AT1g77130.

A database search using the *Arabidopsis* genes AT3g18660 and at1g77130, against the maize database in-house identified four additional sequences which showed significant similarity to the *Arabidopsis* glycogenin genes. The four nucleotide sequences called Maize SEQ 1, Maize SEQ 2, Maize SEQ 3 and Maize SEQ 4 are shown in SEQ ID NOs: 27, 29, 31 and 33 and the deduced amino acid sequences for these nucleotide sequences are shown in SEQ ID NOs: 28, 30, 32 and 34.

Culture conditions

Maize was grown in the greenhouse with a 16 hour daylight and 8 hour night period with a temperature of 24°C during the day and 18°C during the night. Seeds were harvested at different stages between 3 and 35 days after pollination (DAP). Young and medium leaves were also harvested.

Establishment of copy number and identification of glycogenin homolog in maize, wheat and Arabidopsis

Genomic DNA was isolated from *Arabidopsis*, wheat and maize leaves according to the method of Davies *et al.*, ((1994) Methods in Molecular Biology vol. 28: Protocols for nucleic acid analysis by non-radioactive probes, Isaac P.G. (ad) pp 9-15 Humana press, Totowa, NJ USA). DNA was digested with restriction enzyme, EcoRI, XhoI and EcoRV and the digested DNA was run overnight at 20V in 1% agarose gels. The DNA was then transferred to a nylon membrane by vacuum blotting and two identical southern blots were prepared and each one was probed first at a high stringency and later at low stringency conditions. One blot was probed with a digioxygenin labelled AT3g18660 cDNA probe encoding the N-terminus of the gene (a 1.8kb NcoI-AvaI fragment) and filter 2 was probed with AT3g18660 cDNA probe (PGSIP) encoding the C-terminus of the gene (a 700bp C1a K fragment), Figure 5C. Hybridisation was done at 65°C and the blots were first washed with 2 x 5 minutes with 2 x SSC, 0.1 x SDS and later with 0.1 x SSC and 0.1 x SDS at 65°C (high stringency washes). Strong single bands of the expected sizes (5.9kb in the Xho1 cut DNA, 4.6kb in the EcoR1 cut DNA and 5.1kb in the EcoRV cut DNA) were observed only in the lanes containing *Arabidopsis* DNA. No band was observed in the lanes containing maize and wheat DNA, as shown in Fig. 4B. Later the blots were stripped and these were re-probed at 55°C and washed at 60°C for 2 x 15 minutes with 2 x SSC, 0.5 x SDS (low stringency washes). Three bands were observed in the lane containing XhoI digested *Arabidopsis* DNA, two- three bands were observed in the lanes containing maize and wheat DNA, as shown in Fig. 5A and 5B. From the genomic sequence of the AT3g18660 gene it was known that it

spanned two Xho I, EcoR1 and EcoRV sites. This demonstrated that PGSIP exists as a gene family comprising of about 2-3 genes in *Arabidopsis*, maize and wheat.

RNA extraction and first strand cDNA synthesis

Total RNA was extracted from the tissues described above using the method of Napoli *et al* (1990), Plant Cell, 2, 279-289 and in some cases using Qiagen RNA extraction kit following manufacturer s protocol. First strand cDNA was made using SuperscriptII reverse transcriptase (GIBCO-BRL) and oligo dT primer as described in (Frohman *et al*, (1988), Proc. Natl. Acad. Sci. USA, 85:8998):

5′ GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT 3′.

This cDNA pool was used to amplify a maize cDNA homolog to the *Arabidopsis* glycogenin gene (AT3g18660 and at1g77130) utilising the sequence information from the ESTs, GB:BF729544 and GB: BG837930 described above.

EST BF729544 and BG837930 overlapped and these were combined to deduce a single maize PGSIP sequence. Primers were designed to amplify a maize cDNA clone corresponding to this sequence. Primer sequences were as follows.

[GlgmaF] 5'-GGCAATAGAGGAATTCATGTGC-3'
[GlgmaR] 5'-CGTGCAGAACTCGGACCACAG-3'

Construction of a Maize cDNA library

Total RNA was extracted from the various tissues described above (leaves and seeds ranging from 3-35 DAP). The RNA obtained was mixed in equal amounts. This RNA mixture was then used to make a maize cDNA library using SMART cDNA library construction kit (Clontech) following manufacturer's instruction.

Cloning of Maize cDNA

1ul of this first strand cDNA obtained above was used to amplify the cDNA clone represented by the ESTs by PCR using the primers GlgmaF and GlgmaR, the PCR product obtained was cloned into EcoRV cut pBlueScript (SK-) and positive clones were identified. These positive clones were sequenced to confirm that the product obtained indeed represented the sequence in the EST accession number, BF729544. This product was then used to screen the cDNA library and a full length clone was obtained. Similarly a cDNA clone represented by the EST accession no. BG837930 was also cloned.

The PCR conditions were the same as described before for cloning the *Arabidopsis* gene (AT3g18660) of SEQ ID NO: 2.

EXAMPLE 5: cDNA Isolation From Wheat Endosperm

A database search using the *Arabidopsis* genes AT3g18660 and at1g77130, against the wheat in-house database identified one sequence, which showed significant similarity to the *Arabidopsis* PGSIP genes (e-137). The sequence called Wheat SEQ1 is shown in SEQ ID NO: 20.

Culture conditions

Wheat variety NB1 (described in patent WO 00/63398) was grown in the glass house with a 16 hour daylight and 8 hour night period with 22°C during the day and 15°C during the night. Seeds were harvested at different stages between 5 and 20 days after pollination (DAP). Young and medium leaves were also harvested.

RNA extraction and first strand cDNA synthesis

Total RNA was extracted from the above tissues using the method of Napoli et al (1990) and in some cases using Qiagen RNA extraction kit following manufacturer's protocol. First strand cDNA was made using SuperscriptII reverse transcriptase (GIBCO-BRL) and oligo dT primer as described in (Frohman et al, (1988), Proc. Natl. Acad. Sci. USA, 85:8998. This cDNA pool was used to amplify a wheat cDNA homolog to the

Arabidopsis glycogenin gene (AT3g18660 and at1g77130) utilising the sequence information from the maize ESTs, NCBI accession no. BF729544 and BG837930 described above.

Wheat cDNA library making

Total RNA was extracted from the various tissues described above (leaves and seeds ranging from 7-30 days post anthesis (DPA). The RNA obtained was mixed in equal amounts. This RNA mixture was then used to make a wheat cDNA library using SMART cDNA library construction kit (Clontech). Additionally a genomic library from *Triticum tauschii*, var strangulata, accession number CPI 110799, described in (Rahman et al., 1997, Genome, 40:465-474) was also used in this study. The cDNA library from Wheat cv Wyuna described in (Li et al., 1999, Theor. Appl. Gen. 98:226-233) was also used in this study.

Cloning of wheat cDNA

Because a strong band was observed on southern blots probed with the *Arabidopsis* gene (AT3g18660), it was assumed that there is significant degree of homology between the *Arabidopsis*, maize and wheat DNA sequences. A comparison of the *Arabidopsis* and the maize EST sequences also suggested that this was the case. A wheat cDNA library was screened with probes made from the maize and the *Arabidopsis* glycogenin gene. A full length clone was obtained by restriction mapping and analysing the sequence of a number of positive clones.

PCR conditions

The PCR conditions were the same as described before for cloning the *Arabidopsis* gene (AT3gl 8660).

EXAMPLE 6: Agrobacterium Constructs

Construct making

The pSB111 Sulugi described in patent publication WO 00/63398 was used. Six different constructs were made, one each for maize, wheat and *Arabidopsis* in sense orientation and one each for maize, wheat and *Arabidopsis* in antisense orientation for

constitutive expression. Another six set of constructs, were also made using seed specific promoters.

Two constructs were made, one for overexpression and another for downregulation of the Atglycogenin gene. For overexpression, the Atglycogenin gene was excised out from the plasmid (At3g18660 (PGSIP), Figure 1) with SalI-EcoRI digest and ligated in SalI-EcoRI cut pJIT65 resulting in plasmid pCL68. This plasmid was then digested with EcoRI-XhoI and the fragment was ligated into SalI-SmaI cut Nos-NptII SCV resulting in plasmid pCL68 SCV. In this plasmid the Atglycogenin is under 2x 35S promoter for constitutive expression.

For RNAi construct, first a fragment representing the 3' end of the Atglycogenin gene was amplified by PCR using ClaF and Glgstop2 primer (see example 2) and was cloned into pBluescript. The resulting construct was designated pMC167. Clones in both orientation were obtained and the clone with the fragment in reverse orientation was called pMC167inv. pMC167inv was cut with EcoRV-SmaI and ligated back resulting in plasmid pMC167del. pMC167del was cut with HindIII-BamHI and ligated into HindIII-BamHI cut pT7blue2 resulting in plasmid "GlycoinpT7Blue2" (pCL66). Another plasmid (called GlycogeninIRstep1, pCL67) was created by cutting pMC167inv with XhoI-EcoRV and ligating this fragment into XhoI-EcoRV cut pWP446A containing the AtSac25 intron1 . Finally, plasmid "GlycoinpT7Blue2", pCL66 was cut with BamHI-SstI and the fragment ligated into BamHI-SstI cut "GlycogeninIRstep1", pCL67 resulting in plasmid pCL69. pCL69 was cut with EcoRI-XhoI and the fragment was ligated in SCV Nos-NptII at the SmaI-SalI site resulting in plasmid pCL76 SCV. In this plasmid the At glycogenin (PGSIP) RNAi is under 2x35S promoter for constitutive expression.

Figure 6 summarises the whole process and the maps of these plasmids are shown in Figures 9 and 10. The plasmids were transformed into the GV3101 *Agrobacterium* strain and the *Arabidopsis* plants were transformed.

EXAMPLE 7: Transformation of Wheat

Wheat plants transformed with the constructs of Example 6 were produced by the seed inoculation method described in patent publication WO 00/63398. *Solanum tuberosum* c.v. Prairie was transformed with pCL68 SCV and pCL76 SCV using the method of leaf disk

cocultivation essentially as described by Horsch *et al.* (Science 227: 1229-1231, 1985). The youngest two fully-expanded leaves from a 5-6 week old soil grown potato plant were excised and surface sterilised by immersing the leaves in 8% 'Domestos' for 10 minutes. The leaves were then rinsed four times in sterile distilled water. Discs were cut from along the lateral vein of the leaves using a No. 6 cork borer. The discs were placed in a suspension of *Agrobacterium tumefaciens* strain LBA4404 containing one of the two plasmids listed above for approximately 2 minutes. The leaf discs were removed from the suspension, blotted dry and placed on petri dishes (10 leaf discs/plate) containing callusing medium (Murashige and Skoog agar containing 2.5μg/ml BAP, 1 μg/ml dimethylaminopurine, 3% (w/v) glucose). After 2 days the discs were transferred onto callusing medium containing 500μg/ml Claforan and 50μg/ml Kanamycin. After a further 7 days the discs were transferred (5 leaf discs/plate) to shoot regeneration medium consisting of Murashige and Skoog agar containing 2.5μg/ml BAP, 10 μg/ml GA3, 500μg/ml Claforan, 50μg/ml Kanamycin and 3% (w/v) glucose. The discs were transferred to fresh shoot regeneration media every 14 days until shoots appeared. The callus and shoots were excised and placed in liquid Murashige and Skoog medium containing 500μg/ml Claforan and 3% (w/v) glucose. Rooted plants were weaned into soil and grown up under greenhouse conditions to provide tuber material for analysis.

Alternatively, microtubers were produced by taking nodal pieces of tissue culture grown plants onto Murashige and Skoog agar containing 2.5μg/ml Kanamycin and 6% (w/v) sucrose. These were placed in the dark at 19° C for 4-6 weeks when microtubers were produced in the leaf axils.

EXAMPLE 8: Transformation of Maize

Maize plants transformed with the constructs of Example 6 were produced by the seed inoculation method described in patent publication WO 00/63398.

EXAMPLE 9: Transformation of Potato

Transgenic potato plants expressing the *Arabidopsis* plant glycogenin-like gene in sense and antisense orientation were produced.

EXAMPLE 10: Characterisation of the Transgenic Lines

Transgenic plants were analysed by the following methods

For sense constructs, 20 T1 lines were analysed; for antisense constructs, 50 T1 lines were analysed. Plants transformed with sense and antisense sequences of the invention were observed to have altered starch synthesizing ability which was linked to the expression of the transgene.

For the maize, wheat, and potato lines examined, several techniques of analysis were employed. PCR-positive line identification, northern- RNA expression, southern-copy number detection, western-protein expression, amylogenin activity, starch structure and quality, and phenotype all confirmed the successful transformation of the maize, wheat, and potato.

EXAMPLE 11: cDNA Isolation from Rice

The six genes listed in Table 2 were blasted against the rice sequences collected in an in-house database and one new hit was obtained. The accession corresponded to SPTREMBL:Q94HG3, EMBL:AC079633 (SEQ. ID NO: 25) which encodes a protein of 614 AA and shows strong homology to the PGSIP gene (e –129).

EXAMPLE 12: *Arabidopsis* Transformation.

Arabidopsis thaliana c.v. Columbia plants were transformed according to the method of Clough and Brent 1998 Plant J. 16(6):735-743 (1998) with slight modification: Plants were grown to a stage at which bolts were just emerging. Phytagar *0.1%* was added to the seeds and these were vernalized overnight at 4°C. We used 10-15 seeds per 3x5 inch pots. Seed was added onto the soil with a pipette, about 4-5 seeds per ml was dispersed. Seeds were germinated as usual (ie under humidity pots were covered until first leaves appeared and then over a two day period the lid was cracked and then removed). Plants were grown for about 4 weeks in the greenhouse (long day condition) until bolts emerged. The first bolts were cut to encourage growth of multiple secondary bolts. Bolts containing many unopened flower buds were chosen for dipping.

Growing the Agrobacterium culture

Aliquots of the *Agrobacterium* strain GV3101 carrying the constructs pCL68 SCV and pCL68 76 SCV were grown first as a 5ml culture in YEP containing Gentamycin (15ug/ml) and Kanamycin 20ug/ml. Next day, 2ml freshly grown culture was added to 400ml YEP media (10g Yeast Extract, 10g peptone, 5g NaCl, pH 7.0) in a 2 litre flask. and the flask was incubated at 28°C incubator with shaking overnight. Next day OD 600 of the cells was measured and found to be 1.8. Cells were divided into 2X Oakridge bottles and harvested by centrifugation at 5000rpm for 10 min in a GSA rotor at room temperature The pellet was resuspended in 3 volumes of infiltration media so that the final concentration of the culture was 0.6. Infiltration media was prepared by adding the following. ½ Murashige and Skoog Salts, 1x Gamborg's Vitamins and 0.44uM Benzylamino Purine (10ul per L of a 1mg/ml stock), pH was adjusted to 5.7 with NaOH. Then 0.02% Silwet (200ul per 1L) was added and mixed into the solution.

Arabidopsis transformation by Dipping

500 ml of resuspended *Agrobacterium* was poured into a tray and plants were inverted into Agrobacterium solution in batches of 10 for 15 minutes. After 15 minutes the plants were lifted and the excess solution drained, The plants were transferred on their sides to a fresh tray containing tissue paper to allow further soaking of the solution and then transferred to propagating trays. The plants were immediately covered with lids to maintain humidity. After two days the lid was removed and the plants allowed to grow normally. They were not watered for one week until the soil looked dry. After flowereing was complete and the siliques on the plants were dry, all the seeds from one pot were harvested. The seeds were completely dried by keeping harvested seed in an envelope for one week

EXAMPLE 13: Selection of transformed *Arabidopsis thaliana* seed.

Seed produced from transformed *Arabidopsis thaliana* c.v. Columbia plants was weighed into 10 mg aliquots, equivalent to about 500 individual seed, and placed into a sterile 15 ml tube. The seed was surface sterilised by treating with 10 ml of Teepol bleach/ Tween 20 solution (500 ml of 50% (v/v) Teepol bleach containing 1 drop of Tween 20) for five minutes. The seeds were then washed four times with 10ml Tween 20 in sterile water (1 drop Tween 20 in 500ml sterile

water). The seeds were then suspended in 5 ml sterile water and 5ml warm 0.5% agar, mixed carefully and then half of the seeds were spread over one petri dish containing half strength Murashige and Skoog agar medium and the other half over a second dish containing half strength Murashige and Skoog agar medium plus 50 µg/ml kanamycin. The plates were sealed and incubated at 4°C for 48hours. The plates were then transferred to a growth room under low light (2000 lux). Seed on both types of plate germinated but on the plates containing kanamycin non-resistant plants bleached and died within 7 days. Figure 8 demonstrates this selection of kanamycin resistant seedlings. After 14 days the resistant plants were transferred from the selective medium onto MS medium for a further 10 days before being transferred into soil. The plants were grown on to produce leaf material for further analysis.

EXAMPLE 14: Analysis of *Arabidopsis thaliana* Plants Transformed with pCL68 SCV for the Presence of the PGSIP Construct

For the pCL68 SCV transformed lines a total of 31 kanamycin resistant plants were obtained from four of the original floral dips. These were tested for the presence of the construct by PCR.

Genomic DNA extraction

Leaf material was taken from regenerated *Arabidopsis thaliana* plants transformed with pCL68 SCV and genomic DNA isolated. One leaf was excised from a plant growing in soil and placed in a 1.5ml eppendorf tube. The tissue was homogenised using a micropestle and 400µl extraction buffer (200mM Tris HCL pH 8.0; 250mM NaCl; 25mM EDTA; 0.5% SDS) was added and ground again carefully to ensure thorough mixing. Samples were vortex mixed for approximately 5 seconds and then centrifuged at 10,000rpm for 5 minutes. A 350µl aliquot of the resulting supernatant was placed in a fresh eppendorf tube and 350µl chloroform was added. After mixing, the sample was allowed to stand for 5 minutes. This was then centrifuged at 10,000rpm for 5 minutes. A 300µl aliquot of the supernatant was removed into a fresh eppendorf tube. To this was added 300µl of propan-2-ol and mixed by inverting the eppendorf several times. The sample was allowed to stand for 10 minutes. The precipitated DNA was collected by centrifuging at 10,000rpm for 10 minutes. The supernatant was discarded and the pellet air dried.

The pellet of DNA was resuspended in 50µl of distilled water and was used as a template in PCR.

PCR detection of PGSIP

A pair of optimised oligonucleotide primers were designed and synthesised to enable the detection of the pCL68 SCV construct in transformed plants. The sequences of these primers were:

ATGLY002: CGTCTCGTGTCTGGTTTATATTCA

ATGLY003: TCGATGCCTGAGATCTCAGCT

PCR mixtures which contained 5 µl 10x Advantage Taq buffer; 5 µl 2mM dNTPs; 0.5 µl of primer ATGLY002 (100µM); 0.5 µl of primer ATGLY003 (100µM); 5 µl DNA template (*Arabidopsis thaliana* genomic DNA or control pCL68 SCV plasmid DNA); 0.25 µl Advantage Taq polymerase; 33.75 µl distilled water in a final volume of 50µl were set up. The PCR was carried out on a thermocycler using the following parameters: first a hot start at 94°C for 5 min, then 25 cycles consisting of 94° C for 15 sec, 55° C for 30 sec, and 72 °C for 3 min. The cycles were followed by 72 °C for 5 min and a final step of holding the samples at 8 °C.

A diagnostic DNA fragment of 977 bp was produced in these reactions.

The PCR results for pCL68 SCV transformed plants indicated that of the 30 of the 31 of the plants examined had successfully been transformed. Thus, all of the plants except for the plant labeled 1-005 contained the PGSIP gene.

EXAMPLE 15: Analysis of *Arabidopsis thaliana* Plants transformed with pCL76 SCV for the Presence of the PGSIP Downregulation Construct.

For the pCL76 SCV transformed lines a total of 10 kanamycin resistant plants were obtained. Leaf material was taken from regenerated Arabidopsis thaliana plants transformed with pCL76 and genomic DNA isolated. One leaf was excised from a plant growing in soil and placed in a 1.5ml eppendorf tube. The tissue was homogenised using a micropestle and 400µl extraction buffer (200mM Tris HCL pH 8.0; 250mM NaCl; 25mM EDTA; 0.5% SDS)

was added and ground again carefully to ensure thorough mixing. Samples were vortex mixed for approximately 5 seconds and then centrifuged at 10,000rpm for 5 minutes. A 350µl aliquot of the resulting supernatant was placed in a fresh eppendorf tube and 350µl chloroform was added. After mixing, the sample was allowed to stand for 5 minutes. This was then centrifuged at 10,000rpm for 5 minutes. A 300µl aliquot of the supernatant was removed into a fresh eppendorf tube. To this was added 300µl of propan-2-ol and mixed by inverting the eppendorf several times. The sample was allowed to stand for 10 minutes. The precipitated DNA was collected by centrifuging at 10,000rpm for 10 minutes. The supernatant was discarded and the pellet air dried. The pellet of DNA was resuspended in 50µl of distilled water and was used as a template in PCR.

PCR detection of PGSIP RNAi DNA

A pair of optimised oligonucleotide primers were designed and synthesised to enable the detection of the pCL76 SCV construct in transformed plants. The sequences of these primers were:

ATGLY001: TTTGAACAAACAAAAAGGTGGAAC

ATGLY002: CGTCTCGTGTCTGGTTTATATTCA

PCR mixtures which contained 5 µl 10x Advantage Taq buffer; 5 µl 2mM dNTPs; 0.5 µl of primer ATGLY001 (100mM); 0.5 µl of primer ATGLY002 (100mM); 5 µl DNA template (Arabidopsis thaliana genomic DNA or control pCL76 SCV plasmid DNA); 0.25 µl Advantage Taq polymerase; 33.75 µl distilled water in a final volume of 50ml were set up. The PCR was carried out on a thermocycler using the following parameters: first a hot start at 94 C for 5 min, then 25 cycles of 94°C for 15 sec, 55°C for 30 sec, and 72°C for 3 min. The cycles are followed by 72°C for 5 min and the samples are then held at 8°C.

A diagnostic DNA fragment of 819 bp was produced in these reactions.
Out of 8 kanamycin resistant plants tested, 2 were shown to contain the PGSIP RNAi gene construct.

EXAMPLE 16: Constitutive Overexpression and Downregulation of PGSIP Gene in Barley.

Starch is made in the leaves and the grain. To test the effect of overexpressing and downregulating the PGSIP gene in a monocot species, plasmids pCL68 SCV (sense construct) and pCL76 SCV (RNAi construct) were expressed in barley. These plasmids conferred constitutive expression as the genes were under the control of the double 35S promoter. Additionally, the full length gene and the RNAi cassette were expressed under the control of the rice actin promoter (US patent number 56141876). For this purpose, the Gateway cloning technology was used according to manufacturers instruction with slight modification (Invitrogen). The full length PGSIP was excised from plasmid pMC168 with NcoI-EcoRI and cloned into pENTR4 vector cut with NcoI-EcoRI resulting in plasmid called pMC175. The RNAi cassette was excised from plasmid pCL76 SCV with SalI-EcoICRI and cloned into pENTR1 vector cut with SalI-EcoRV resulting in plasmid pMC174. These plasmids were then recombined with Destination vector pWP492R12 SCV that contained the actin promoter flanked by two recombination sites (attR1 and attR2 on either side (Invitrogen). This resulted in plasmids pMC177 and pMC176 respectively which contained the PGSIP gene and the RNAi construct under the control of the rice actin promoter (US patent number 56141876). These plasmids are shown in Figs. 9 and 10.

The constructs were transformed into Agrobacterium strain (AGL-1) (Lazo et al., 1991, Bio/Technol 9: 963–967) for barley transformation. Immature embryos of the barley variety Golden Promise were transformed essentially according to the method of Tingay et al. (The Plant Journal 11(6): 1369-1376, 1997). Donor plants of Golden Promise were grown with an 18 hours day, and 18/13°C. Immature embryos (1.5 - 2.0 mm) were isolated and the axes removed. They were then dipped into an overnight liquid culture of Agrobacterium, blotted and transferred to co-cultivation medium. After 2 days the embryos were transferred to MS based callus induction medium with Asulam and Timentin for 10 days. Tissues were transferred at 2 weekly intervals, and at each transfer they were cut into small pieces and lined out on the plate. At the third transfer, only the embryogenic tissue was moved on to fresh medium. After a total of 8 weeks in culture, the tissue was transferred to regeneration

medium (FHG), where plantlets formed within 2 - 4 weeks. These were transferred to Beatsons jars with growth regulator free medium until roots had formed, when they were transferred to Jiffies expandable teat pellets and then to the Conviron growth chambers.

The plants were analysed by PCR using following primers.

For plants containing pCL68 plasmid (sense expression)

5-' ATTTGGAGAGGACAGCCCAAGC Glyc For

5'- CTCCATCGTTGGATCTCGTTCG-3' Glyc Rev (S)

For plants containing pCL76 plasmid (RNAi expression)

5'-ATTTGGAGAGGACAGCCCAAGC-3' Glyc For

5'-GCGTCATCTTCATCGCCAATCC – 3' Glyc Rev (D)

PCR was carried out as described in above

Results:

Six barley plants were regenerated after transformation with plasmid pCL68 SCV and eight plants with plasmid pCL76 SCV. The plants were first analysed by PCR and the leaves of the positive plants were subjected to iodine staining by Lugol. The results of PCR analysis are presented in Table 7.

Table 7. results of PCR screen of barley plants transformed with pCL68 SCV or pCL76 SCV.

Construct	Plant no	PCR no.	PCR
	Control1	GG11	Neg
	Control2	GG12	Neg
	Control3	GG13	Neg
pCL68	1	GG1	Pos
pCL68	2	GG2	Neg

pCL68	3		
pCL68	4.1	GG8	Neg
pCL68	5.1		
pCL68	6.1	GG3	Neg
pCL68	6.2		
pCL68	6.3	GG9	Neg
pCL68	7.1	GG10	Neg
pCL76	1.1	GG4	Pos
pCL76	1.2	GG5	Pos
pCL76	1.3	GG6	Pos
pCL76	1.4	GG14	Pos
pCL76	1.5	GG15	Neg
pCL76	2	GG7	Neg
pCL76	3.1	GG16	Pos
pCL76	4.1	GG17	Neg

One plant containing the sense construct was found to contain more starch granules in its leaves relative to control plants without the sense construct. The plants containing the RNAi construct were found to lack starch granules as shown in Figure 11A.

EXAMPLE 17: Seed Specific Overexpression and Downregulation of the PGSIP Gene in Barley

For seed specific expression, the plasmids pMC174 and pMC175 were recombined with the plasmid pWP491R12SCV that contained the seed specific promoter flanked by two recombination sites (attR1 and attR2 on either side (Invitrogen)). Barley plants were transformed according to the method of Tingay et al. (1997) with some modification as described for Example 13.

EXAMPLE 18: Analysis of Transformed *Solanum tuberosum* Plants for Presence of the PGSIP Construct

Analysis of regenerated Potato transformants.

Leaf material was taken from regenerated potato plants and genomic DNA isolated. One large potato leaf (approximately 30mg) was excised from an *in vitro* grown plant and placed in a 1.5ml eppendorf tube. The tissue was homogenised using a micropestle and 400µl extraction buffer (200mM Tris HCL pH 8.0; 250mM NaCl; 25mM EDTA; 0.5% SDS) was added and ground again carefully to ensure thorough mixing. Samples were vortex mixed for approximately 5 seconds and then centrifuged at 10,000rpm for 5 minutes. A 350µl aliquot of the resulting supernatant was placed in a fresh eppendorf tube and 350µl chloroform was added. After mixing, the sample was allowed to stand for 5 minutes. This was then centrifuged at 10,000rpm for 5 minutes. A 300µl aliquot of the supernatant was removed into a fresh eppendorf tube. To this was added 300µl of propan-2-ol and mixed by inverting the eppendorf several times. The sample was allowed to stand for 10 minutes. The precipitated DNA was collected by centrifuging at 10,000rpm for 10 minutes. The supernatant was discarded and the pellet air dried. The pellet of DNA was resuspended in 50µl of distilled water and was used as a template in PCR.

PCR mixtures which contained 5 µl 10x Advantage Taq buffer; 5 µl 2mM dNTPs; 0.5 µ l of either primer ATGLY001 or ATGLY003 (100µM); 0.5 µl of primer ATGLY002 (100µM); 5 µl DNA template (*Solanum tuberosum* c.v. Prairie genomic DNA, control pCL68 SCV plasmid DNA or control pCL76 SCV plasmid DNA); 0.25 µl Advantage Taq polymerase; 33.75 µl distilled water in a final volume of 50µl were set up. The PCR was carried out on a thermocycler using the following parameters: first a hot start at 94°C for 5 min, followed by 25 cycles of 94 ° C for 15 sec, 55° C for 30 sec, and 72 ° C for 3 min. The cycles were followed by 72 ° C for 5 min and a finally holding the temperature at 8 ° C.

A diagnostic DNA fragment of 977 bp was produced in these reactions from plasmid pCL68 SCV or 819 bp from plasmid pCL76 SCV. Lines of *Solanum tuberosum* c.v. Prairie transformed with pCL68 SCV or pCL76 SCV were tested by PCR and were shown to contain the construct.

Of 18 plants transformed with pCL68 SCV, all 18 contained the sense PGSIP construct. For the PGSIP RNAi construct (pCL76 SCV), 3 out of 8 plants contained the construct.

EXAMPLE 19: Analysis of Transformed Plants for PGSIP Expression.

Raising antisera to PGSIP proteins.

Expression of PGSIP proteins can be analysed by Western blotting. Antibodies to PGSIP are raised by inoculating rabbits with peptides corresponding to the *Arabidopsis thaliana* PGSIP protein sequences produced by expressing the sequence as a transcriptional fusion with glutathione-S-transferase in *E. coli* cells

Preparation of protein extracts.

Protein extracts from potato tuber were produced by taking up to 100mg of tissue and homogenising in 1ml of ice cold extraction buffer consisting of 50mM HEPES pH 7.5, 10mM EDTA, 10mM DTT. Additionally, protease inhibitors, such as PMSF or pepstatin were included to limit the rate of protein degradation. The extract was centrifuged at 13000 rpm for 1 minute and the supernatant decanted into a fresh eppendorf tube and stored on ice. The supernatants was assayed for soluble protein content using, for example, the BioRad dye-binding protein assay (Bradford, M.C. (1976) Anal. Biochem. 72, 248-254).

An aliquot of the soluble protein sample, containing between 10-50μg total protein was placed in an eppendorf tube and excess acetone (ca 1.5ml) added to precipitate the proteins which were collected by centrifuging the sample at 13000 rpm for 5 minutes. The acetone was decanted and the samples air-dried until all the residual acetone has evaporated.

SDS-polyacrylamide gel electrophoresis.

The protein samples were separated by SDS-PAGE. SDS PAGE loading buffer (2% (w/v) SDS; 12% (w/v) glycerol; 50 mM Tris-HCl pH 8.5; 5 mM DTT; 0.01% Serva blue G250) was added to the protein samples (up to 50 l). Samples were heated at 70°C for 10 minutes before loading onto a NuPage polyacrylamide gel. The electrophoresis conditions were 200 V constant for 1 hour on a 10% Bis-Tris precast polyacrylamide gel, using 50 mM MOPS, 50 mM Tris, 1 mM EDTA, 3.5 mM SDS, pH 7.7 running buffer, according to the NuPage methods

(Invitrogen, US 5,578,180).

Electroblotting.

Separated proteins were transferred from the acrylamide gel onto PVDF membrane by electroblotting (Transfer buffer: 20% methanol; 25 mM Bicine pH 7.2; 25 mM Bis-Tris, 1 mM EDTA, 50 _M chlorobutanol) in a Novex blotting apparatus at 30 V for 1.5 hours.

Immunodetection.

After blocking the membrane with 5% milk powder in Tris buffered saline (TBS-Tween) (20mM Tris, pH 7.6; 140mM NaCl; 0.1% (v/v) Tween-20), the membrane was challenged with a rabbit anti-PGSIP antiserum at a suitable dilution in TBS-Tween. Specific cross-reacting proteins were detected using an anti-rabbit IgG-Horse radish peroxidase conjugate secondary antibody and visualised using the enhanced chemiluminescence (ECL) reaction (Amersham Pharmacia).

Detection of mRNA.

Expression of PGSIP mRNA was analysed in plants by rtPCR or by Northern blotting.

EXAMPLE 20: Analysis of Leaf Starch Content

Samples of leaves from control and transformed *Arabidopsis thaliana* plants which had been grown for 24 hours under high light (about 60 mg) were taken in a microfuge tube and extracted with 100 µl of 45% $HClO_4$. This suspension was diluted with 1 ml of distilled water and centrifuged (14000 rpm, 2 min.) Aliquots of the extracts were then analysed for starch content by taking 100 µl of the extract and mixing with an equal volume of Lugol's solution, the optical density of which was then measured at 540nm using a microplate reader. Standard starch mixtures were prepared in the same way and measured at the same time and the starch content of the extracts was calculated by reference to these standards.

Table 8. Starch contents of leaves of *Arabidopsis thaliana* plants transformed with pCL68 SCV (sense construct comprising SEQ ID NO: 1) compared with the starch contents of leaves of non

transformed (ncc) control plants. Control value is the mean ± (the standard error of the mean) for three plants.

samples	leaf starch content ug/g fresh weight (FWt).
37256	19.95
1-002	12.68
1-003	49.68
1-004	48.02
1-005	13.88
37407	17.47
37437	49.55
37468	24.88
37499	8.65
37529	17.71
37560	15.93
37590	9.95
37621	6.02
37257	21.9
37288	18.20
37316	11.82
37261	22.85
37381	9.51
37412	13.21
37442	33.60
37473	17.96
37504	8.88
37534	18.58
37565	11.98

37295	32.83
37323	38.43
37354	16.16
ncc	22.59 (±5.08)

The ncc value represents the mean and standard error for the three control plants. Each data point otherwise represents a single leaf from an individual plant. Taking the error of the control as a measure of the population variation, then plants 1-003, 1-004, 1-007, 1-008, 6-007 and 9-003 have significantly more starch in their leaves than the controls. Plants 1-009, 1-012, 1-013, 2-003, 6-005, 6-009 and 6-011 have significantly lower starch contents. The copy number and level of expression of the sense construct in the plants are to be determined. The results demonstrate that a sense construct comprising SEQ ID NO: 1 can effectively alter the content of starch.

Table 9. Starch contents of leaves of *Arabidopsis thaliana* plants transformed with pCL76 SCV (RNAi construct) compared to controls.

Samples		starch content µg per leaf
pCL76 SCV	7	27.20
pCL76 SCV	20.1	26.96
Control	ncc	42.97

The data in these tables shows that the leaves of the transformed plants have an altered starch content compared to the untransformed controls (ncc).

EXAMPLE 21: Microscopic Analysis of Starch Granule Size and Number.

Starch granules were extracted from *Arabidopsis thaliana* or *Solanum tuberosum* tissue by taking 50-100 mg of tissue and homogenising in 1% sodium metabisulphite solution. After filtering the extract through miracloth, the starch was collected by centrifugation, 1300rpm for 5 minutes and then resuspended in 1 ml of water. Aliquots were taken and an equal amount of Lugol solution added to enhance the contrast of the starch granules. Suspensions were prepared for microscope imaging by placing onto a microscope slide. Representative micrographs were taken of the samples. The electronically captured images were then processed using suitable image analysis software, such as the package 'ImageJ'. This enabled a quantification of the size distributions of different starch samples to be made and compared.

Alternatively, samples of purified starch are either suspended in water and viewed with a light microscope or sputter -coated with gold and viewed with a scanning electron microscope such as a Phillips (Eindhoven, The Netherlands) XL30 Field Emission Gun scanning electron microscope at 3kV.

Starch granules can be examined in tissues as well. For example, starch in tissues is stained using Lugol's solution (1% Lugol's solution, I-KI [1:2, v/v]; Merck). Starch can then be examined, for example, in longitudinal sections of tubers. Alternatively the starch can be further isolated subsequent to staining and suspended in water, and stained again with a few drops of Lungol's solution and examined microscopically.

The radii of the blue staining core of the starch granules and the total granule are measured microscopically using an ocular micrometer. If granules are ovoid in shape, both long radius and short radius measurements are taken. The radii of the blue-staining core and the total granule are determined by measuring individual, randomly chosen starch granules.

EXAMPLE 22: Analysis of Starch Functionality.

Preparation of starch.

Starch was extracted from potato tubers by taking 0.5-1 kg of washed tuber tissue and homogenising using a juicerator chased with 200ml of 1% Sodium bisulphite solution. The starch was allowed to settle, the supernatant decanted off and the starch washed by

resuspending in 200 ml of ice-cold water. The resulting starch pellet was left to air dry. Once dried the starch was stored at -20 C.

Alternatively, other methods can be utilized to isolate starch, for example, samples of tubers are first homogenized in extraction buffer (10 mM EDTA, 50 mM Tris, pH 7.5, 1mM DTT, 0.1% Na2S2O5). The resulting fibrous substance is then washed several times with the extraction buffer and filtered. The filtrate is allowed to set at 4 °C and the supernatant is discarded after the starch granules have settled. Starch granules are then washed with extraction buffer, water, and acetone and dried at 4 °C.

With maize and other cereal crops, seeds are soaked in 50ml of a 20 mM sodium acetate, pH 6.5, 10 mM mercuric chloride solution. After 24 hr, the germ and pericarp are removed and 50 ml of fresh solution is added for an additional 24 hr. Endosperm is repeatedly homogenized for 1 minute intervals in a mortar and pestle, and freed starch granules are purified by multiple extractions with saline and toluene (Boyer et al., 1976, Cereal Chemistry 53: 327-337). Granular starch is washed three times with double distilled water, once with acetone, and dried at 40 °C.

Viscometric analysis of starch.

Starch samples were analysed for functionality by testing rheological properties using viscometric analysis (rapid visco analyzer (RVA) or differential scanning calorimetry (DSC)). Viscosity of starches can also be measured by various other techniques. For example, a Rapid Visco Analyser Series 4 instrument (Newport Scientific, Sydney Australia) can be utilized with a 13 min profile where 2 g of starch are analyzed in water at a concentration of 7.4% (w/v) and the analysis used the stirring and heating protocol that suggested by Newport Scientific. For longer profiles, 2.5 g starch samples are used at a concentration of 10% (w/v). The sample is heated while stirring at 1.5 °C min^{-1} from 50 °C to 95 °C for 15 min then cooled to 50 °C at 15 min^{-1}. Viscosity is measured in centipose (cP).

EXAMPLE 23: Analysis of Fine Structure of Starch

Amylopectin chain length distribution

One method for examining the fine structure of starch is ^{14}C labeling of amylopectin chains to determine chain lengths. Extracted starch granules are suspended at 25 mg ml^{-1} in medium comprising 100 mM Bicine (pH 8.50, 25 mM potassium acetate, 10 mM DTT, 5 mM EDTA, 1 mM ADP[U-^{14}C] glucose at 18.5 GBq mol^{-1} and 10 µl starch suspension in a total volume of 100 µl, for each sample. Samples are then incubated for 1 hour at 25 °C. The incubation is terminated by addition of 3 ml 750 ml^{-1} aqueous methanol containing 10 g l-1 KCL (methanol/KCL). After incubation for at least 5 minutes at room temperature, starch is collected by centrifugation at 2000 g for 5 min. The supernatant is disgarded and the pellet is resuspended in 0.3 ml distilled water. The Methanol/KCL wash, centrifugation, and resuspension are repeated 2-4 times. The resulting pellets are dried at room temperature, dissolved with 50 µl 1M NaOH, and diluted with 50 µl distilled water. To determine the average length of amylopectin chains into which ^{14}C was incorporated, products of incubation with ADP[U-^{14}C] glucose are debranched with isoamylase and subjected to chromatography on a column of Sepharose CL-4B. The glucan eluding earlier from the column consists of longer chains than glucan eluding later from the column.

Another method for examining the fine structure of starch is chromatography without labeling. A 10 mg sample of isolated starch is dissolved in 100 ul 0.1 M NaOH for 1 hour at 95 °C. The sample is diluted in 900 µl water, 150 µl 1 M soduim citrate (pH 5.0). The starch is then debranched by adding 300 units of isoamylase, or hydrolysed with 300 units of alpha-amylase, or beta-amylase for 24 hours at 37 °C. A 100 ul aliquot sample of the hydrolysed samples is analyzed with chromatography. For example HPAE-PAD chromatography (Carbo PAC PA-100 column; Dionex, Idstein, Germany; flow 1 ml min^{-1}; buffer A: 150 mM NaOH; buffer B: 1 M sodium acetate in buffer A) with an applied gradient comprising 0-5 min 100% A; 5-20 min 85% A, 15% B, 20-35 min 70% A, 30% B (linear); 35-80 min 50% A, 50% B (convex).

Alternatively, HPLC chromatography is utilized, where partially hydrolyzed debranched starch samples in 0.01 N NaOH (5 mg/ml), and 2 ml are applied to a size exclusion column (Sephadex G-75, 1.5 X 100cm). The mobile phase is 0.01 N NaOH and

the flow rate is 0.6-0.9 ml/min. Samples are analyzed for total carbohydrate by the phenol-sulfuric acid test (Hodge and Hofreiter, 1962,Vol. 1, R.L. Whistler and ML Wolform (Eds.), Corporation. Version 7. Academic Press, New York, pp: 388-389) and the Park Johnson test for reduced ends (Porro et al., 1981, Anal Biochem. 118(2):301-6). Based on these to analyses the average chain length for each fraction is calculated.

Amylopectin is further characterized by measuring the low molecular weight to high molecular weight chain ratio (on a weight basis) according to the method of Hizukuri (Hizukuri, 1986, Carbohydrate Research, 147, 342–347).

An alternative method for analyzing amylopectin chains is gel electrophoresis. Starch samples are debranched with isoamylase, derivatised with fluorophore APTS, and subjected to gel electrophoresis in an Applied Biosystem DNA sequencer. Data are analized by Genescan software. The method allows for identification of authentic maltohexaose and maltoheptaose as well as a determination of percent molar differences and the degree of polymerization, distribution of chain lengths, between samples.

Amylose content of starch

Amylose percentages are determined by gel permeation chromatography according to Denyer et al. (Denyer et al., 1995, Plant Cell Environ 18:1019-1026) or by gel filtration analysis according to Boyer and Liu (Boyer and Liu, 1985, Starch Starke 37:73-79).

Alternatively, the amylose contents are determined spectrophotometrically in 1 to 2 mg isolated starch according to the iodometric method described by Hovenkamp-Hermelink et al. 1988. Amperometric titrations are performed according to Williams et al 1970 to determine the average amylose content per sample.

EXAMPLE 24: cDNA Isolation From Barley

A database search using the *Arabidopsis* genes AT3g18660 and at1g77130, against an in-house database identified two barley sequences. The accessions corresponding to Genbank: BE438665 and Genbank: BE438754 showed significant similarity to the *Arabidopsis* PGSIP genes (9e –34). The sequences called Barley SEQ1 and Barley SEQ2 are shown in SEQ ID Nos: 16 and 18.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference

CLAIMS

1. An isolated nucleic acid molecule that:

(i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, or a fragment thereof;

(ii) comprises a nucleotide sequence at least 40% identical to SEQ ID NOs: 1 or 2, or a complement thereof; or

(iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 1 or 2 under low stringency conditions of hybridization, or a complement thereof.

2. The isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises SEQ ID NOs: 1 or 2 or a complement thereof.

3. The isolated nucleic acid molecule of claim 1, comprising a nucleotide sequence selected from the group consisting of nucleotide residues 516-592, 681 to 918, 1039 to 1655, 1762 to 2536, and 2991 to 3264 of SEQ ID NO: 1.

4. An isolated nucleic acid molecule that:

(i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 11, or a fragment thereof;

(ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NO: 10, or a complement thereof, wherein the nucleotide sequence does not encode the amino acid of SEQ ID NO: 35; or

(iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NO: 10 under stringent conditions of hybridization, or a complement thereof, wherein the nucleotide sequence does not encode the amino acid of SEQ ID NO: 35.

5. The isolated nucleic acid molecule of claim 4, wherein the nucleic acid molecule comprises SEQ ID NO: 10 or a complement thereof.

6. An isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence that is at least 98% identical to SEQ ID NO: 9.

7. An isolated nucleic acid molecule thereof comprising the nucleotide sequence of SEQ ID NO: 8 or a complement thereof.

8. An isolated nucleic acid molecule that:

(i) comprises a nucleotide sequence which encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof;

(ii) comprises a nucleotide sequence at least 70% identical to SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof; or

(iii) hybridizes to a nucleic acid molecule consisting of SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33 under stringent conditions of hybridization, or a complement thereof.

9. The isolated nucleic acid molecule of claim 8, wherein the nucleic acid molecule comprises SEQ ID NOs: 4, 5, 6, 12, 14, 16, 18, 20, 23, 25, 27, 29, 31, 33, or a complement thereof.

10. A fragment of the isolated nucleic acid molecule of any one of claims 1-9, wherein the fragment comprises at least 40, 60, 80, 100 or 150 contiguous nucleotides of the nucleic acid molecule.

11. The isolated nucleic acid molecule of claim 1 comprising the nucleotide sequence of nucleotides 1-195 of SEQ ID NO: 2, or a complement thereof.

12. An isolated polypeptide comprising the amino acid sequence of amino acid residues 1-65 of SEQ ID NO: 3, or a fragment thereof.

13. An isolated polypeptide comprising:

(i) an amino acid sequence that is at least 70% identical to SEQ ID NO: 3 or a fragment thereof;

(ii) an amino acid sequence encoded by the nucleic acid molecule of claim 1; or

(iii) an amino acid sequence of SEQ ID NO: 3.

14. An isolated polypeptide comprising:

(i) an amino acid sequence at least 70% identical to SEQ ID NO: 11, or a fragment thereof;

(ii) an amino acid sequence encoded by the nucleic acid molecule of claim 4; or

(iii) an amino acid sequence of SEQ ID NO: 11.

15. An isolated polypeptide comprising:

(i) an amino acid sequence that is at least 98% identical to SEQ ID NO: 9;

(iii) an amino acid sequence encoded by the nucleic acid molecule of SEQ ID NO: 8, or a complement thereof; or

(v) an amino acid sequence of SEQ ID NO: 9, or a fragment thereof.

16. An isolated polypeptide comprising:

(i) an amino acid sequence that is at least 70% identical to SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34, or a fragment thereof;

(ii) an amino acid sequence encoded by the nucleic acid molecule of claim 8;

(iii) an amino acid sequence of SEQ ID NOs: 7, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34.

17. A fragment of a polypeptide comprising at least 5 amino acid residues, wherein said fragment is a portion of the polypeptide encoded by a nucleic acid molecule selected from the group consisting of exon I, exon II, exon III, exon IV and exon V of SEQ ID NO: 1.

18. A polypeptide comprising the amino acid sequence of SEQ ID: 3, 7, 9, 11, 13, 15, 17, 19, 21, 22, 24, 26, 28, 30, 32, 34 which further comprising one or more conservative amino acid substitution.

19. A fusion protein comprising the amino acid sequence of any one of claims 12-18 and a heterologous polypeptide.

20. A fragment or immunogenic fragment of a polypeptide of any one of claims 12-18, wherein the fragment comprises at least 5, 8, 10, 15, 20, 25, 30 or 35 consecutive amino acids of the polypeptide.

21. An antibody that immunospecifically binds to a polypeptide of any one of the claims 12-18.

22. A method for making a polypeptide of any one of the claims 12-18, comprising the steps of:

(a) culturing a cell comprising a recombinant polynucleotide encoding the polypeptide of any one of claims 12-18 under conditions that allow said polypeptide to be expressed by said cell; and

(b) recovering the expressed polypeptide.

23. A complex comprising a polypeptide encoded by a nucleic acid molecule of any of claims 1-9 and a starch molecule.

24. The complex of claim 23, wherein the starch molecule comprises from 1 to 700 glucose units.

25. The complex of claim 23, wherein the starch molecule comprises branching chains of glucose polysaccharides.

26. A vector comprising a nucleic acid molecule of any one of claims 1-9.

27. An expression vector comprising a nucleic acid molecule of any one of claims 1-9 and at least one regulatory region operably linked to the nucleic acid molecule.

28. The expression vector of claim 27, wherein the regulatory region confers chemically-inducible, dark-inducible, developmentally regulated, developmental-stage specific, wound-induced, environmental factor-regulated, organ-specific, cell-specific, and/or tissue-specific expression of the nucleic acid molecule or constitutive expression of the nucleic acid molecule.

29. The expression vector of claim 27, wherein the regulatory region is selected from the group consisting of a 35S CaMV promoter, a rice actin promoter, a patatin promoter, and a high molecular weight glutenin gene of wheat.

30. An expression vector comprising the antisense sequence of a nucleic acid molecules of any one of claims 1-9, wherein the antisense sequence is operably linked to at least one regulatory region.

31. A genetically-engineered cell which comprises a nucleic acid molecule of any one of claims 1-9.

32. A cell comprising the expression vector of claim 27.

33. A cell comprising the expression vector of claim 30.

34. A genetically-engineered plant comprising the isolated nucleic acid molecule of any of claims 1-9.

35. The genetically-engineered plant of claim 34 and progeny thereof, further comprising a transgene encoding an antisense nucleotide sequence.

36. The genetically-engineered plant of claim 31, further comprising an RNA interference construct.

37. A cell comprising an a 35SCaMV constitutive promoter operably linked to a nucleic acid molecule of SEQ ID NO:2 or a rice actin promoter operably linked to an RNA interference construct comprising fragments of a nucleic acid molecule of SEQ ID NO:2, wherein said promoter confers expression of said fragments.

38. A method of altering starch synthesis in a plant comprising introducing into a plant:

(i) a nucleic acid sequence comprising a starch primer gene, or a fragment thereof;

(ii) a nucleotide sequence that hybridises under stringent conditions to a sequence of (i) or its complement; or

(iii) an agent which is capable of altering the expression of a sequence of (i) or (ii);

such that starch synthesis is altered relative to a plant without any of the above sequences.

39. A method of altering starch synthesis in a plant comprising, introducing into a plant an expression vector of claim 27, such that starch synthesis is altered relative to a plant without the expression vector.

40. A method of altering starch synthesis in a plant comprising, introducing into a plant at least an expression vector of claim 30, such that starch synthesis is altered in comparison to a plant without the expression vector.

41. A method of altering starch granules in a plant comprising, introducing into a plant at least an expression vector of claim 27, such that the starch granules are altered in comparison to a plant without the expression vector.

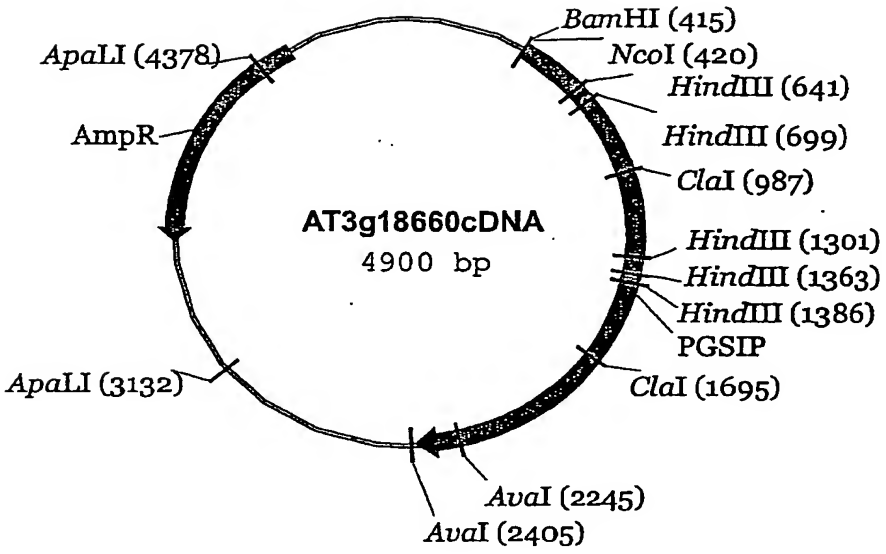
42. A method of altering starch granules in a plant comprising, introducing into a plant at least an expression vector of claim 30, such that the starch granules are altered in comparison to a plant without the expression vector.

43. The method of claim 42, wherein starch granules are absent from leaves of the plant comprising at least an expression vector.

44. A plant part comprising a nucleic acid molecule of any of claims 1-9 or a nucleic acid of the method of claim 38, wherein starch synthesis is altered.

45. The plant part of claim 44, wherein the part is a tuber, seed or leaf.

46. The modified starch obtained from the plant parts of claim 44, wherein the modification is selected from the group consisting of a ratio of amylose to amylopectin, amylose content, size of starch granules, quantity of size of starch granules, a ratio of small to large starch granules, and rheological properties of the starch as measured using viscometric analysis.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NcoI (420)
HindIII (641)
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HindIII (1363)
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PGSIP
ClaI (1695)
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Figure 1

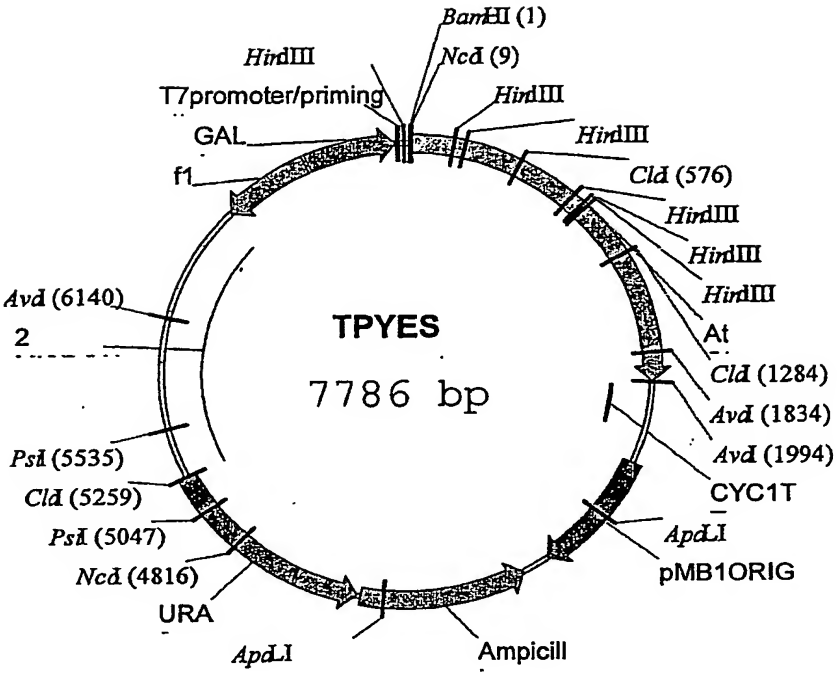


Figure 2

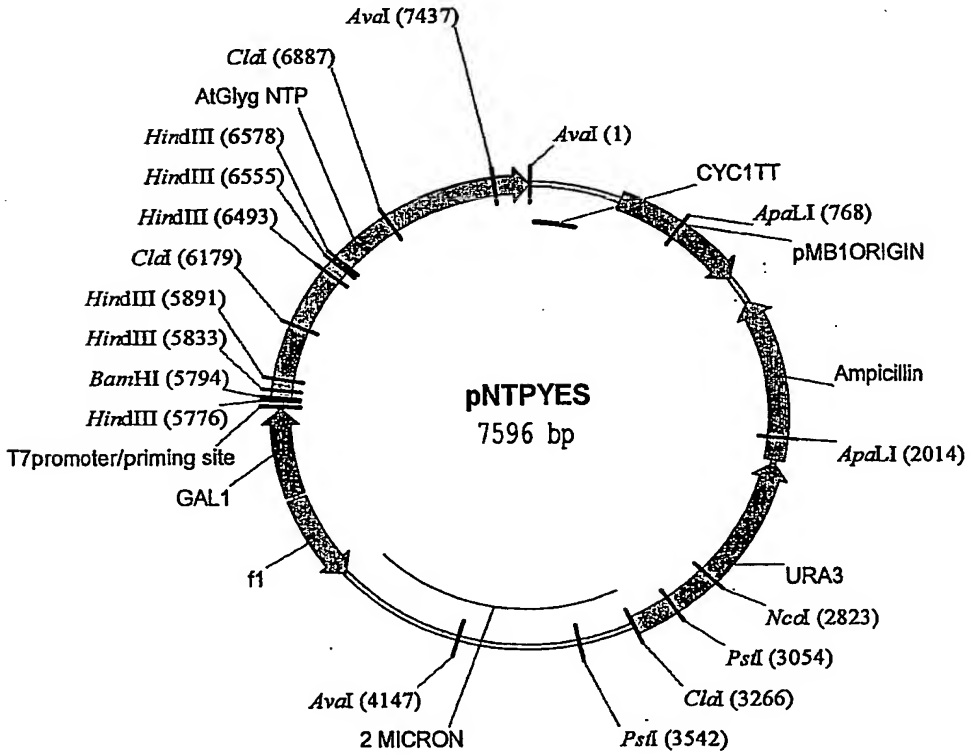


Figure 3

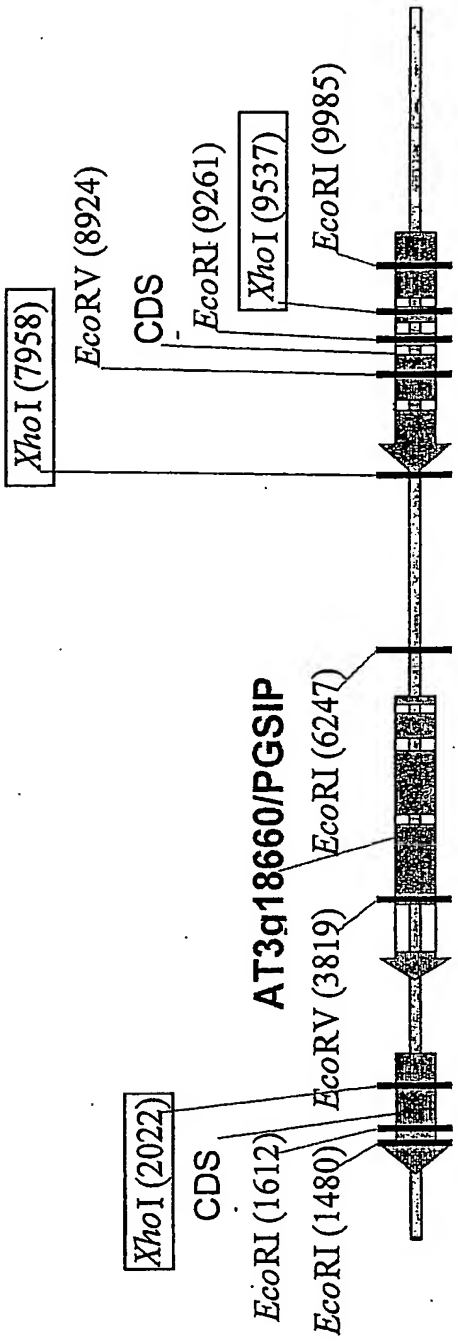

XhoI (7958)
EcoRV (8924)
CDS
EcoRI (9261)
XhoI (9537)
EcoRI (9985)
AT3g18660/PGSIP
EcoRI (6247)
EcoRV (3819)
XhoI (2022)
CDS
EcoRI (1612)
EcoRI (1480)

Figure 4A

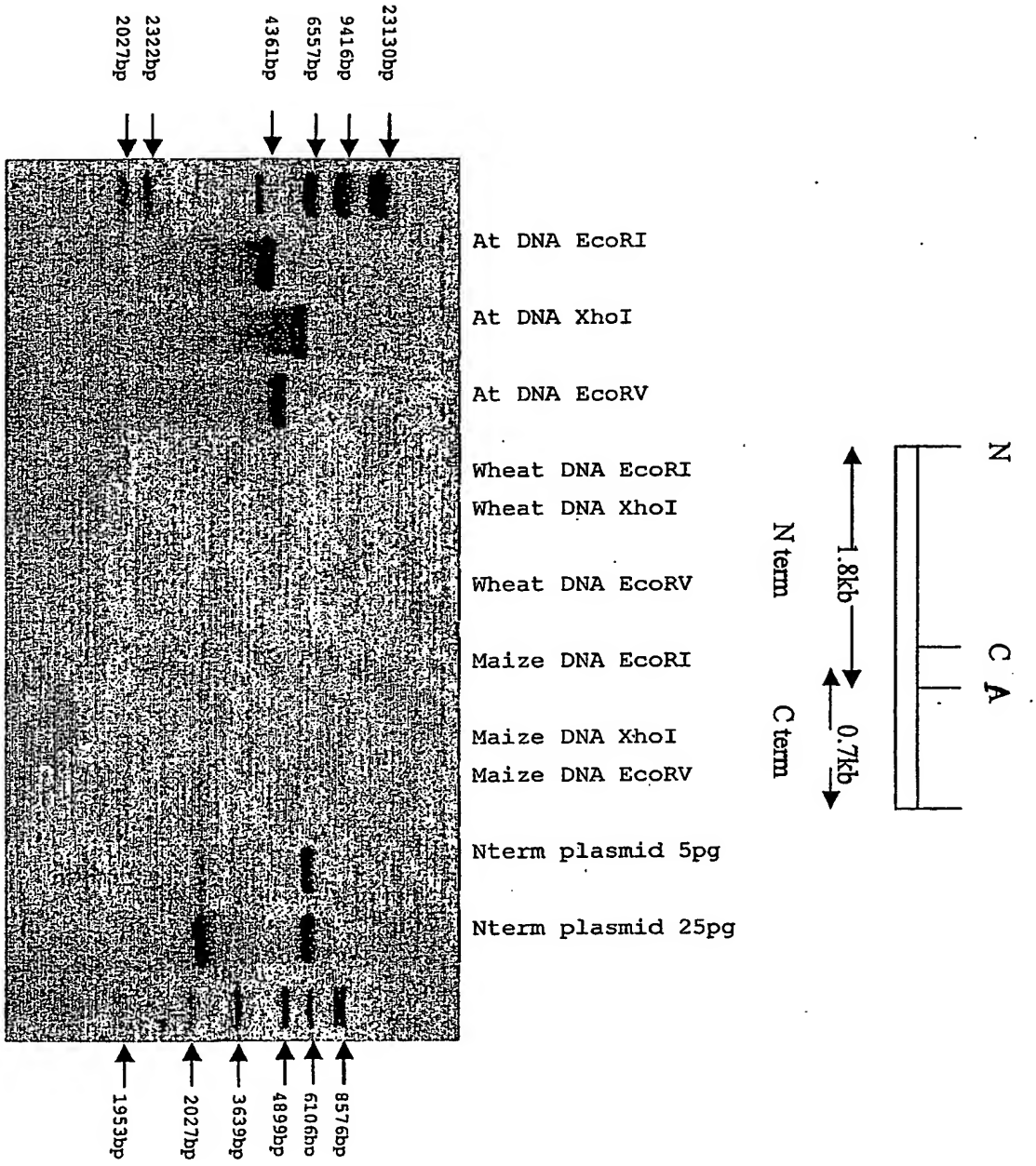


Figure 4B

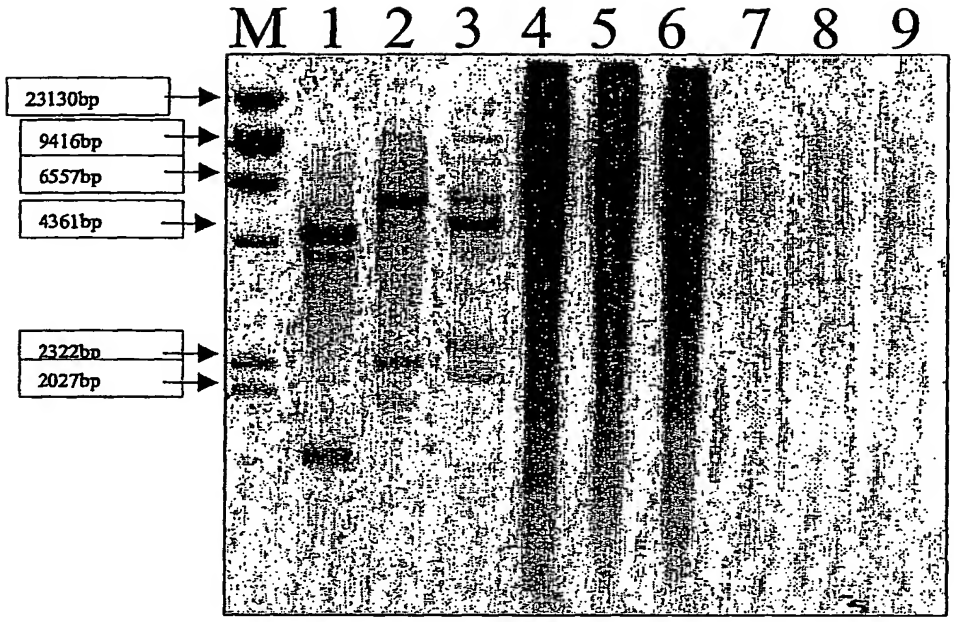


Figure 5A

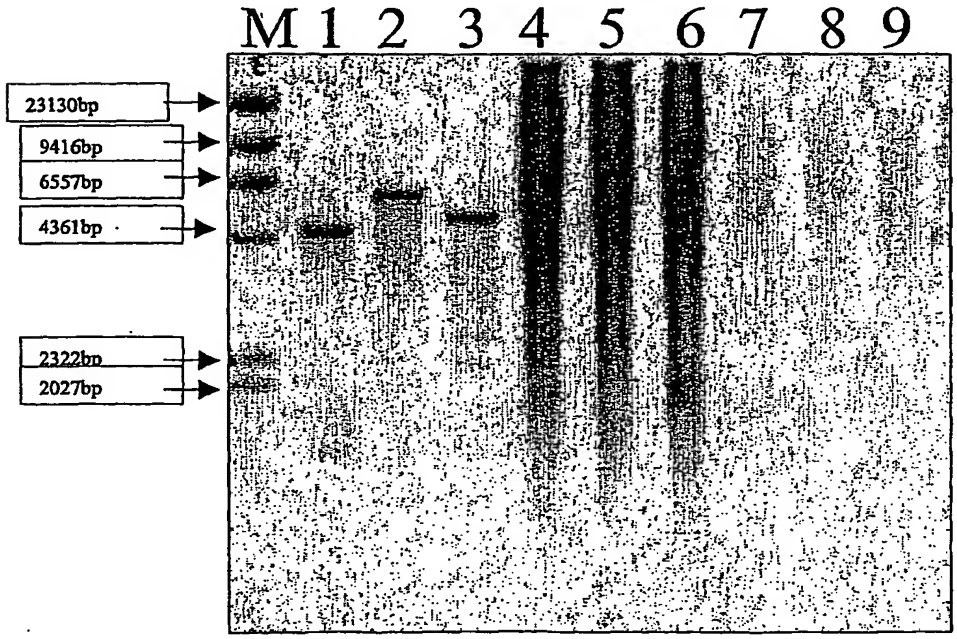


Figure 5B

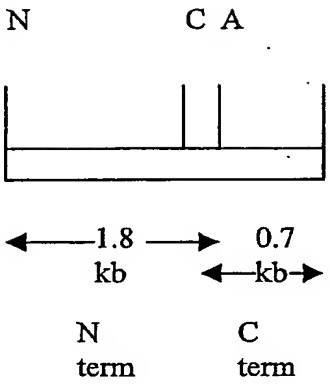
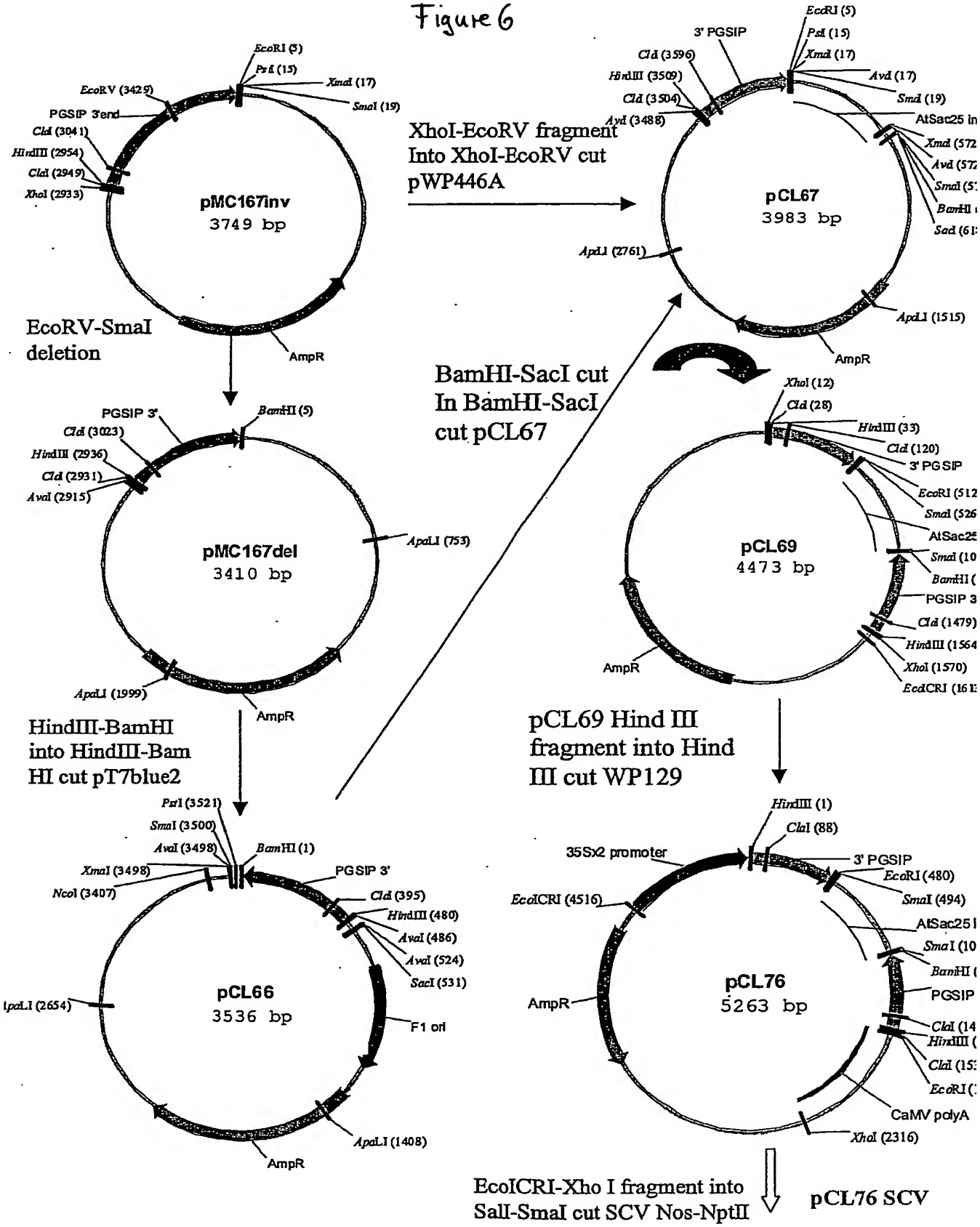


Figure 5C

Figure 6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PstI (15)
XmaI (17)
SmaI (19)
EcoRV (3429)
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HindIII (2954)
ClaI (2949)
XhoI (2933)
AmpR
EcoRV-SmaI deletion
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PGSIP 3'
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ClaI (3023)
HindIII (2936)
ClaI (2931)
AvaI (2915)
ApaLI (753)
ApaLI (1999)
AmpR
HindIII-BamHI into HindIII-Bam HI cut pT7blue2
pCL66
3536 bp
PstI (3521)
SmaI (3500)
AvaI (3498)
BamHI (1)
XmaI (3498)
NcoI (3407)
PGSIP 3'
ClaI (395)
HindIII (480)
AvaI (486)
AvaI (524)
SacI (531)
F1 ori
ApaLI (1408)
AmpR
XhoI-EcoRV fragment Into XhoI-EcoRV cut pWP446A
pCL67
3983 bp
EcoRI (5)
3' PGSIP
PstI (15)
ClaI (3596)
XmaI (17)
HindIII (3509)
AvaI (17)
ClaI (3504)
SmaI (19)
AvaI (3488)
AtSac25 in
ApaLI (2761)
ApaLI (1515)
AmpR
BamHI-SacI cut In BamHI-SacI cut pCL67
pCL69
4473 bp
XhoI (12)
ClaI (28)
HindIII (33)
ClaI (120)
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EcoRI (512
SmaI (526
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SmaI (10
BamHI (
PGSIP 3
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XhoI (1570)
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AmpR
pCL69 Hind III fragment into Hind III cut WP129
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ClaI (88)
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EcoICRI (4516)
SmaI (494)
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BamHI (
PGSIP
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HindIII (
ClaI (15
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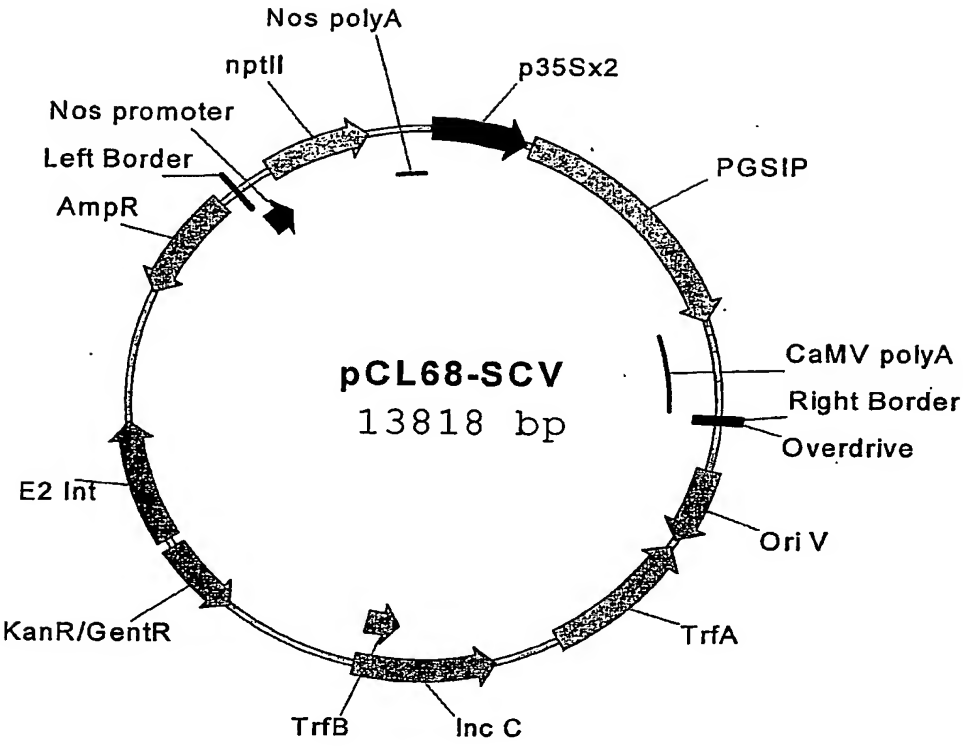

Figure 7

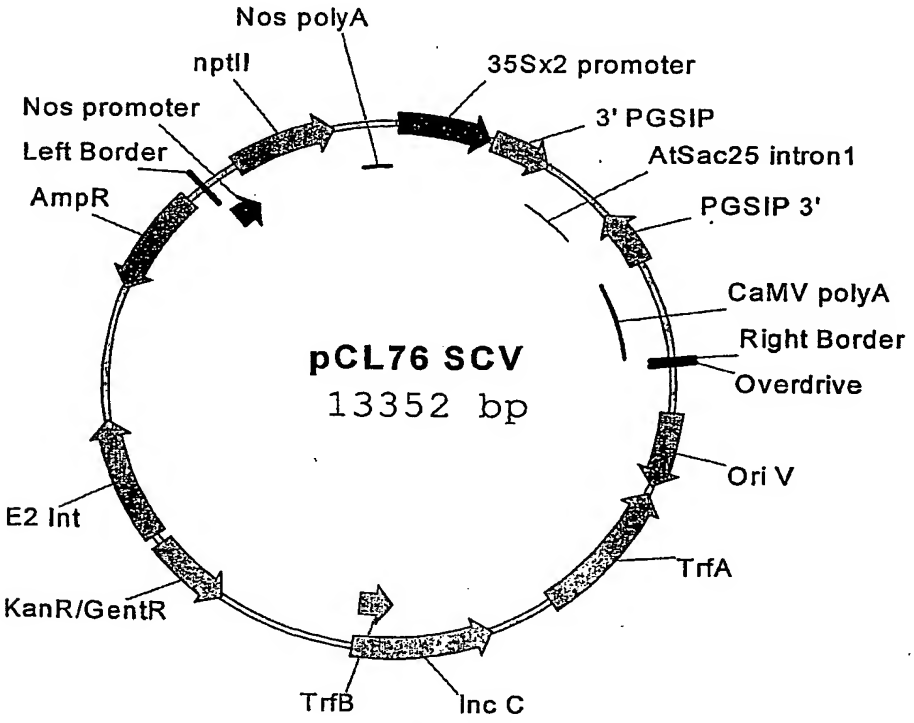

Figure 8

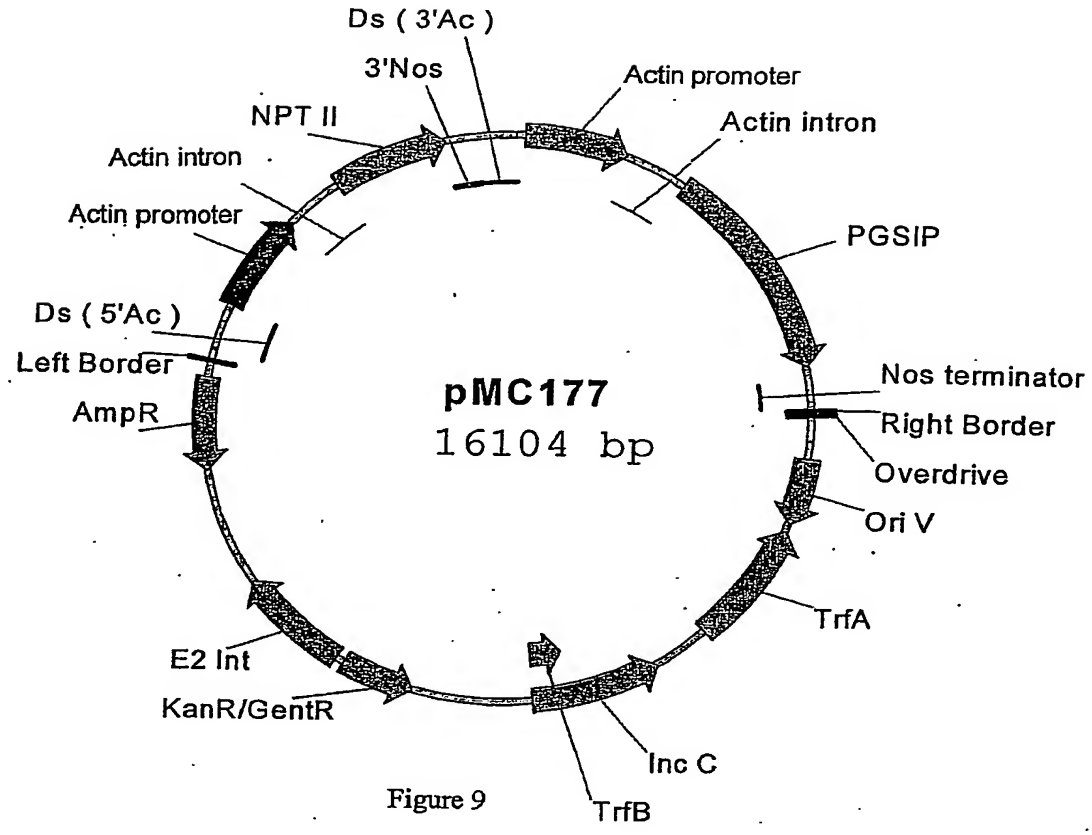

Figure 9

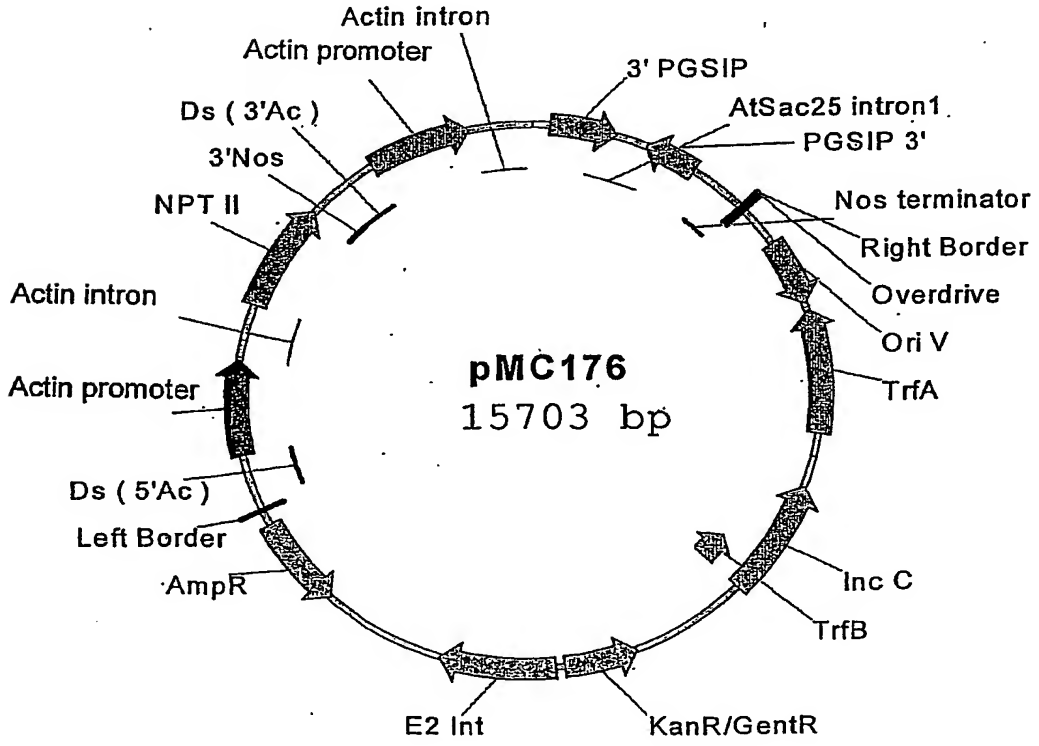

Figure 10

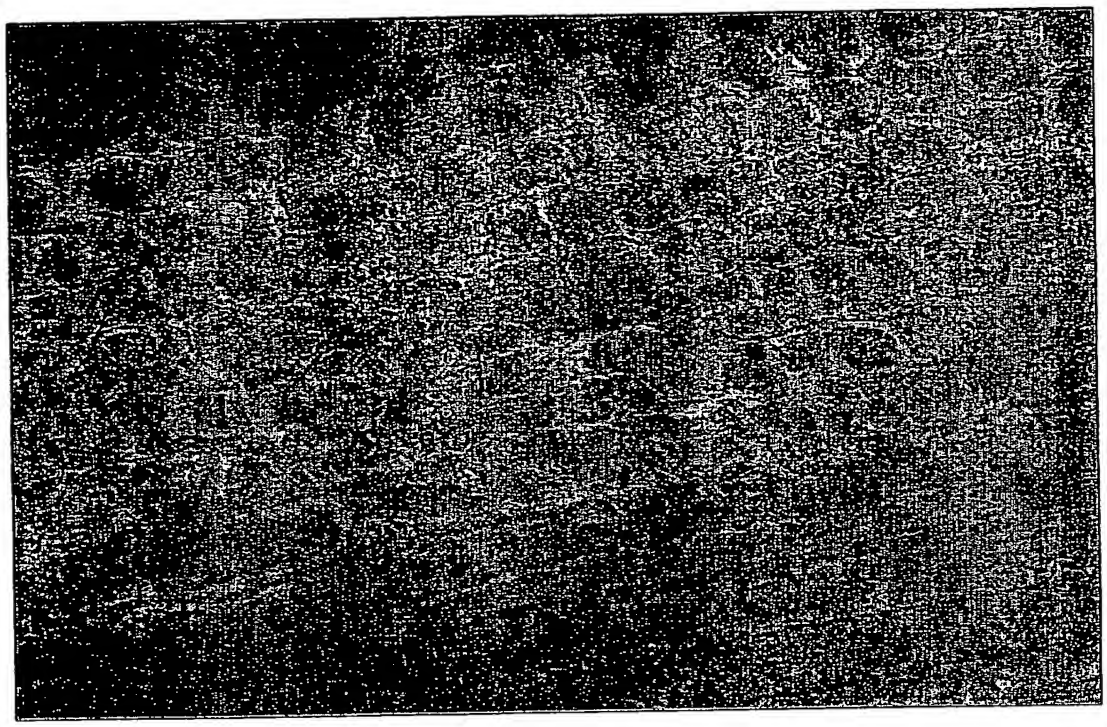

Figure 11A.

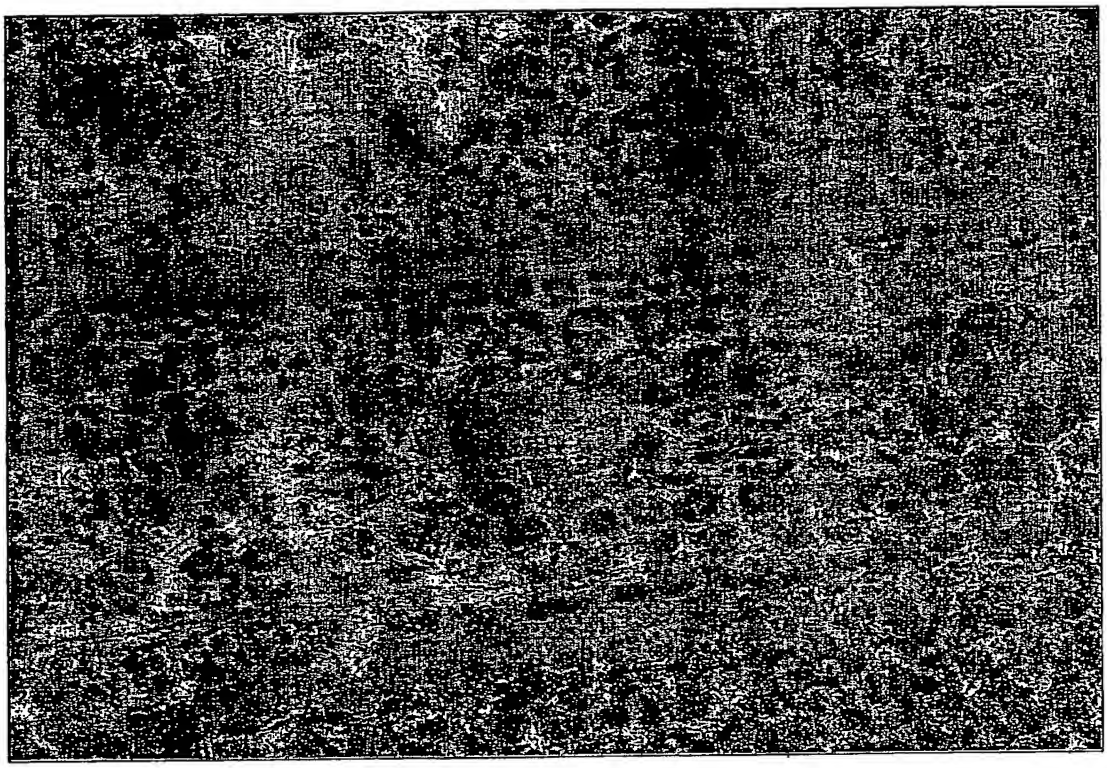

Figure 11B.

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His Ser Leu Ser His Ser Ser Ser Arg Trp Ile Trp Arg Arg Gln Asp
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acc ctt gag aac atc gaa gaa ggc cgt acg atc ggt gtc ttg aat ttt   432
Thr Leu Glu Asn Ile Glu Glu Gly Arg Thr Ile Gly Val Leu Asn Phe
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Asn Gly Asp Glu Glu Lys Val Val Val Leu Asn Leu Asp Tyr Ala Asp
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Gln Glu Thr Glu Val Pro Val Cys Pro Asn Ile Pro Asn Ile Lys Val
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Pro Arg Phe Lys Ile Cys Ile Asp Lys Leu Cys Asn Trp Lys Ser Met
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Leu Arg His Trp Gly Glu Ser Asn Trp Thr Asp Tyr Glu Ser Phe Val
625                 630                 635                 640
Pro Thr Pro Pro Ala Ile Thr Val Asp Arg Arg Ser Ser Leu Pro Gly
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His Asn Leu
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ctgtgagctt gctgttccac tcaacgcaaa agcacgactc tacacggtag acagacgcag 240
agaagcatat gctacaatac tgcattcagc aagtgaatat gtttgcggtg cgataacagc 300
agctcaaagc attcgtcaag caggatcaac aagagacctt gttattcttg ttgatgacac 360
cataagtgac taccaccgca aggggctgga atctgctggg tggaaggtta gaataataca 420
gaggatccgg aatcccaaag cggaacgtga tgcctacaac gaatggaact acagcaaatt 480
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atgccggaga tcgccgcgac ggscaacaac gccacgctct tcaactccgg cgtcatggtc 180
gtcgagccct ccaactgcac gttccgcctg ctcatggacc acatcgacga gatcacctcg 240

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tacaacggcg gggaccaggg gtacctcaac gagatattca cgtggtggca ccgcgtcccc 300
aggcacatga acttcctcaa gcacttctgg gagggcgaca gcgaggccat gaaggcgaag 360
aagacacagc tgttcggcgc ggacccgccg gtcctctacg tcctccacta ccttggcctc 420
aagccgtggc tgtgcttcag agactacgac tgcaactgga acaacgccgg gatgcgcgag 480
ttcgccagcg acgtcgcgca tgcccggtgg tggaaggtgc acgacaggat gccccggaag 540
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caggccgaga aggccaactc tcaagatggc cactggcgcc tcaacgtcac ggacaccagg 660
ctcaagacgt gctttgagaa gttctgcttc tgggagagca tgctctggca ttggggcgag 720
aacagtaaca ggaccaagag cgtccccatg gcagccacga cggcaaggtc gtgatctgta 780
gatatacgaa caccccatcc ccatatggca accatacatg catagcaata gcttgtatag 840
gtagctatgc tttagttctt cgctatatat acagaataca ccactcgatc cctgttgttg 900
tcaaggctgc agctctatgt cgctgccggc ctgccaccat ggctaacgat tcttttgggt 960
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<400> 6

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Met Cys Val Asn Phe Ser Ser Leu Lys Leu Val Leu Phe Leu Met Met
  1               5                  10                  15

ctg gtt gct atg ttc aca ctc tac tgt tct cca ccg ttg caa att cct   96
Leu Val Ala Met Phe Thr Leu Tyr Cys Ser Pro Pro Leu Gln Ile Pro
             20                  25                  30

gaa gat cca tca agt ttt gca aac aaa tgg ata cta gaa cct gct gta  144
Glu Asp Pro Ser Ser Phe Ala Asn Lys Trp Ile Leu Glu Pro Ala Val
         35                  40                  45

acc aca gat cct cgc tat ata gct aca tct gag atc aac tgg aac agt  192
Thr Thr Asp Pro Arg Tyr Ile Ala Thr Ser Glu Ile Asn Trp Asn Ser
     50                  55                  60

atg tca ctt gtt gtt gag cat tac tta tct ggc aga agc gag tat caa  240
Met Ser Leu Val Val Glu His Tyr Leu Ser Gly Arg Ser Glu Tyr Gln
 65                  70                  75                  80

gga att ggc ttt cta aat ctc aac gat aac gag att aat cga tgg cag  288
Gly Ile Gly Phe Leu Asn Leu Asn Asp Asn Glu Ile Asn Arg Trp Gln
                 85                  90                  95

gtg gtc ata aaa tct cac tgt cag cat ata gct ttg cat cta gac cat  336
Val Val Ile Lys Ser His Cys Gln His Ile Ala Leu His Leu Asp His
```

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                100                 105                 110

gct gca agt aac ata act tgg aaa tct tta tac ccg gaa tgg att gac   384
Ala Ala Ser Asn Ile Thr Trp Lys Ser Leu Tyr Pro Glu Trp Ile Asp
        115                 120                 125

gag gaa gaa aaa ttc aaa gtc ccc act tgt cct tct ctt cct tgg att   432
Glu Glu Glu Lys Phe Lys Val Pro Thr Cys Pro Ser Leu Pro Trp Ile
    130                 135                 140
caa gtt cct gac aag tct cga atc gat ctt atc att gcc aag ctc cca   480
Gln Val Pro Asp Lys Ser Arg Ile Asp Leu Ile Ile Ala Lys Leu Pro
145                 150                 155                 160

tgt aac aag tca gga aaa tgg tca aga gat gtg gct aga ttg cac tta   528
Cys Asn Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu
                165                 170                 175

caa ctt gca gca gct cga gtg gcg gca tct tct gaa ggg ctt cat gat   576
Gln Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Glu Gly Leu His Asp
            180                 185                 190

gtt cat gtg att ttg gta tca gat tgc ttt cca ata ccg aat ctt ttt   624
Val His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe
        195                 200                 205

acg ggt caa gaa ctt gtt gcc cgt caa gga aac ata tgg ctg tat aag   672
Thr Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys
    210                 215                 220

cct aaa ctt cac cag tta aga caa aag tta caa ctt cct gtt ggt tcc   720
Pro Lys Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser
225                 230                 235                 240

tgt gaa ctt tct gtt cct ctt caa gct aaa gat aat ttc tac tcg gca   768
Cys Glu Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala
                245                 250                 255

aat gcc aag aaa gaa gcg tac gcg acg atc ttg cac tca gat gat gct   816
Asn Ala Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Asp Asp Ala
            260                 265                 270

ttt gtc tgt gga gcc att gca gta gca cag agc att cga atg tca ggc   864
Phe Val Cys Gly Ala Ile Ala Val Ala Gln Ser Ile Arg Met Ser Gly
        275                 280                 285

tct act cgc aat ttg gta ata cta gtc gat gat tcg atc agt gaa tac   912
Ser Thr Arg Asn Leu Val Ile Leu Val Asp Asp Ser Ile Ser Glu Tyr
    290                 295                 300

cat aga agt ggc ttg gaa tca gct gga tgg aag att cac aca ttt caa   960
His Arg Ser Gly Leu Glu Ser Ala Gly Trp Lys Ile His Thr Phe Gln
305                 310                 315                 320
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aga atc aga aac ccg aaa gct gaa gca aat gca tat aac caa tgg aac   1008
Arg Ile Arg Asn Pro Lys Ala Glu Ala Asn Ala Tyr Asn Gln Trp Asn
                325                 330                 335

tac agc aaa ttc cgt ctt tgg gaa ttg aca gaa tac aac aag atc atc   1056
Tyr Ser Lys Phe Arg Leu Trp Glu Leu Thr Glu Tyr Asn Lys Ile Ile
            340                 345                 350

ttc att gat gca gac atg ctt atc ctc aga aac atg gat ttc ctc ttc   1104
Phe Ile Asp Ala Asp Met Leu Ile Leu Arg Asn Met Asp Phe Leu Phe
        355                 360                 365

gag tac ccc gaa atc tcc aca act gga aac gac ggt acg ctc ttc aac   1152
Glu Tyr Pro Glu Ile Ser Thr Thr Gly Asn Asp Gly Thr Leu Phe Asn
    370                 375                 380

tcc ggt cta atg gtg att gaa cca tca aat tca aca ttc cag tta cta   1200
Ser Gly Leu Met Val Ile Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu
385                 390                 395                 400

atg gat cac atc aac gat atc aat tcc tac aat gga gga gac caa ggt   1248
Met Asp His Ile Asn Asp Ile Asn Ser Tyr Asn Gly Gly Asp Gln Gly
                405                 410                 415

tac ctt aac gag ata ttc aca tgg tgg cat cgg att cca aaa cac atg   1296
Tyr Leu Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met
            420                 425                 430

aat ttc ttg aag cat ttc tgg gaa gga gac aca cct aag cac agg aaa   1344
Asn Phe Leu Lys His Phe Trp Glu Gly Asp Thr Pro Lys His Arg Lys
        435                 440                 445

tct aag acg aga cta ttt gga gct gat cct ccg ata ctc tac gtt ctt   1392
Ser Lys Thr Arg Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu
    450                 455                 460

cat tac cta ggt tac aac aaa cca tgg gta tgc ttc aga gac tac gat   1440
His Tyr Leu Gly Tyr Asn Lys Pro Trp Val Cys Phe Arg Asp Tyr Asp
465                 470                 475                 480

tgc aat tgg aat gtc gtt gga tac cat caa ttc gcg agc gat gaa gca   1488
Cys Asn Trp Asn Val Val Gly Tyr His Gln Phe Ala Ser Asp Glu Ala
                485                 490                 495

cac aaa act tgg tgg aga gtg cac gac gcg atg cct aag aaa ttg cag   1536
His Lys Thr Trp Trp Arg Val His Asp Ala Met Pro Lys Lys Leu Gln
            500                 505                 510

agg ttt tgt cta ctg agt tcg aaa caa aag gcg caa ctt gag tgg gat   1584
Arg Phe Cys Leu Leu Ser Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp
        515                 520                 525
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cgg aga caa gct gag aaa gcg aat tac aga gac gga cat tgg agg att   1632
Arg Arg Gln Ala Glu Lys Ala Asn Tyr Arg Asp Gly His Trp Arg Ile
    530                 535                 540

aag atc aaa gat aag aga ctt acg act tgt ttt gaa gat ttc tgt ttc   1680
Lys Ile Lys Asp Lys Arg Leu Thr Thr Cys Phe Glu Asp Phe Cys Phe
545                 550                 555                 560

tgg gag agt atg ctt tgg cat tgg ggc gat tat gaa att ctc gaa acc   1728
Trp Glu Ser Met Leu Trp His Trp Gly Asp Tyr Glu Ile Leu Glu Thr
                565                 570                 575

gac cct ggt ctt acg gag acg atg ata cct tcc tca agt ccc atg gag   1776
Asp Pro Gly Leu Thr Glu Thr Met Ile Pro Ser Ser Ser Pro Met Glu
            580                 585                 590

tca aga cat cga ctc tcg ttc tca aat gag aag aca agt agg agg aga   1824
Ser Arg His Arg Leu Ser Phe Ser Asn Glu Lys Thr Ser Arg Arg Arg
        595                 600                 605

ttt caa aga att gag aag ggt gtc aag ttc aac act ctg aaa ctt gtg   1872
Phe Gln Arg Ile Glu Lys Gly Val Lys Phe Asn Thr Leu Lys Leu Val
    610                 615                 620

ttg att tgt ata atg ctt gga gct ttg ttc acg atc tac cgt ttt cgt   1920
Leu Ile Cys Ile Met Leu Gly Ala Leu Phe Thr Ile Tyr Arg Phe Arg
625                 630                 635                 640

tat cca ccg cta caa att cct gaa att cca act agt ttt ggt ctt act   1968
Tyr Pro Pro Leu Gln Ile Pro Glu Ile Pro Thr Ser Phe Gly Leu Thr
                645                 650                 655

act gat cct cgc tat gta gct aca gct gag atc aac tgg aac cat atg   2016
Thr Asp Pro Arg Tyr Val Ala Thr Ala Glu Ile Asn Trp Asn His Met
            660                 665                 670

tca aat ctt gtt gag aag cac gta ttt ggt aga agc gag tat caa gga   2064
Ser Asn Leu Val Glu Lys His Val Phe Gly Arg Ser Glu Tyr Gln Gly
        675                 680                 685

att ggt ctt ata aat ctt aac gat aac gag att gat cga ttc aag gag   2112
Ile Gly Leu Ile Asn Leu Asn Asp Asn Glu Ile Asp Arg Phe Lys Glu
    690                 695                 700

gta acg aaa tct gac tgt gat cat gta gct ttg cat cta gat tat gct   2160
Val Thr Lys Ser Asp Cys Asp His Val Ala Leu His Leu Asp Tyr Ala
705                 710                 715                 720

gca aag aac ata aca tgg gaa tct tta tac ccg gaa tgg att gat gaa   2208
Ala Lys Asn Ile Thr Trp Glu Ser Leu Tyr Pro Glu Trp Ile Asp Glu
                725                 730                 735
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gtt gaa gaa ttc gaa gtc cct act tgt cct tct ctg cct ttg att caa   2256
Val Glu Glu Phe Glu Val Pro Thr Cys Pro Ser Leu Pro Leu Ile Gln
            740                 745                 750

att cct ggc aag cct cgg att gat ctt gta att gcc aag ctt ccg tgt   2304
Ile Pro Gly Lys Pro Arg Ile Asp Leu Val Ile Ala Lys Leu Pro Cys
        755                 760                 765

gat aaa tca gga aaa tgg tct aga gat gtg gct cgc ttg cat tta caa   2352
Asp Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu Gln
    770                 775                 780

ctt gca gca gct cga gtg gcg gct tct tct aaa gga ctt cat aat gtt   2400
Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Lys Gly Leu His Asn Val
785                 790                 795                 800

cat gtg att ttg gta tct gat tgc ttt cca ata ccg aat ctt ttt acg   2448
His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe Thr
                805                 810                 815

ggt caa gaa ctt gtt gcc cgt caa gga aac ata tgg ctg tat aag cct   2496
Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys Pro
            820                 825                 830

aat ctt cac cag cta aga caa aag tta cag ctt cct gtt ggt tcc tgt   2544
Asn Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser Cys
        835                 840                 845

gaa ctt tct gtt cct ctt caa gct aaa gat aat ttc tac tcc gca ggt   2592
Glu Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala Gly
    850                 855                 860

gca aag aaa gaa gct tac gcg act atc ttg cat tct gcc caa ttt tat   2640
Ala Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Gln Phe Tyr
865                 870                 875                 880

gtc tgt gga gcc att gca gct gca cag agc att cga atg tca ggc tct   2688
Val Cys Gly Ala Ile Ala Ala Ala Gln Ser Ile Arg Met Ser Gly Ser
                885                 890                 895

act cgt gat ctg gtc ata ctt gtt gat gaa acg ata agc gaa tac cat   2736
Thr Arg Asp Leu Val Ile Leu Val Asp Glu Thr Ile Ser Glu Tyr His
            900                 905                 910

aaa agt ggc ttg gta gct gct gga tgg aag att caa atg ttt caa aga   2784
Lys Ser Gly Leu Val Ala Ala Gly Trp Lys Ile Gln Met Phe Gln Arg
        915                 920                 925

atc agg aac ccg aat gct gta cca aat gcc tac aac gaa tgg aac tac   2832
Ile Arg Asn Pro Asn Ala Val Pro Asn Ala Tyr Asn Glu Trp Asn Tyr
    930                 935                 940
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agc aag ttt cgt ctt tgg caa ctg act gaa tac agt aag atc atc ttc   2880
Ser Lys Phe Arg Leu Trp Gln Leu Thr Glu Tyr Ser Lys Ile Ile Phe
945                 950                 955                 960

atc gat gca gac atg ctt atc ctg aga aac att gat ttc ctc ttc gag   2928
Ile Asp Ala Asp Met Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Glu
                965                 970                 975

ttc cct gag ata tca gca act gga aac aat gct acg ctc ttc aac tct   2976
Phe Pro Glu Ile Ser Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser
            980                 985                 990

ggt cta atg gtg gtt gag cca tct aat tca aca ttc cag tta cta atg   3024
Gly Leu Met Val Val Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu Met
        995                1000                1005

gat aac att aat gaa gtt gtg tct tac aac gga gga gac caa ggt tac   3072
Asp Asn Ile Asn Glu Val Val Ser Tyr Asn Gly Gly Asp Gln Gly Tyr
    1010                1015                1020

ctt aac gag ata ttc aca tgg tgg cat cgg att cca aaa cac atg aat   3120
Leu Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn
1025                1030                1035                1040

ttc ttg aag cat ttc tgg gaa gga gac gaa cct gag att aaa aaa atg   3168
Phe Leu Lys His Phe Trp Glu Gly Asp Glu Pro Glu Ile Lys Lys Met
                1045                1050                1055

aag acg agt cta ttt gga gct gat cct ccg atc cta tac gtt ctt cat   3216
Lys Thr Ser Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu His
            1060                1065                1070

tac cta ggt tat aac aaa ccc tgg tta tgc ttc aga gac tat gac tgc   3264
Tyr Leu Gly Tyr Asn Lys Pro Trp Leu Cys Phe Arg Asp Tyr Asp Cys
        1075                1080                1085

aat tgg aat gtc gat att ttc cag gaa ttt gct agt gac gag gct cat   3312
Asn Trp Asn Val Asp Ile Phe Gln Glu Phe Ala Ser Asp Glu Ala His
    1090                1095                1100

aaa acc tgg tgg aga gtg cac gac gca atg cct gaa aac ttg cat aag   3360
Lys Thr Trp Trp Arg Val His Asp Ala Met Pro Glu Asn Leu His Lys
1105                1110                1115                1120

ttc tgt cta cta aga tcg aaa cag aag gcg caa ctt gaa tgg gat agg   3408
Phe Cys Leu Leu Arg Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp Arg
                1125                1130                1135

aga caa gca gag aaa ggg aac tac aaa gat gga cat tgg aag ata aag   3456
Arg Gln Ala Glu Lys Gly Asn Tyr Lys Asp Gly His Trp Lys Ile Lys
            1140                1145                1150
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atc aaa gac aag aga ctt aag act tgt ttc gaa gat ttc tgc ttt tgg   3504
Ile Lys Asp Lys Arg Leu Lys Thr Cys Phe Glu Asp Phe Cys Phe Trp
       1155                1160                1165

gag agt atg ctt tgg cat tgg ggt gag acg aac tct acc aac aat tct   3552
Glu Ser Met Leu Trp His Trp Gly Glu Thr Asn Ser Thr Asn Asn Ser
   1170                1175                1180

tcc acc acc acc act tca tca ccg ccg cat aaa acc gct ctc cct tcc   3600
Ser Thr Thr Thr Thr Ser Ser Pro Pro His Lys Thr Ala Leu Pro Ser
1185                1190                1195                1200

ctg tga                                                          3606
Leu
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            20                  25                  30
Glu Asp Pro Ser Ser Phe Ala Asn Lys Trp Ile Leu Glu Pro Ala Val
        35                  40                  45
Thr Thr Asp Pro Arg Tyr Ile Ala Thr Ser Glu Ile Asn Trp Asn Ser
    50                  55                  60
Met Ser Leu Val Val Glu His Tyr Leu Ser Gly Arg Ser Glu Tyr Gln
65                  70                  75                  80
Gly Ile Gly Phe Leu Asn Leu Asn Asp Asn Glu Ile Asn Arg Trp Gln
                85                  90                  95
Val Val Ile Lys Ser His Cys Gln His Ile Ala Leu His Leu Asp His
            100                 105                 110
Ala Ala Ser Asn Ile Thr Trp Lys Ser Leu Tyr Pro Glu Trp Ile Asp
        115                 120                 125
Glu Glu Glu Lys Phe Lys Val Pro Thr Cys Pro Ser Leu Pro Trp Ile
    130                 135                 140
Gln Val Pro Asp Lys Ser Arg Ile Asp Leu Ile Ile Ala Lys Leu Pro
145                 150                 155                 160
Cys Asn Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu
                165                 170                 175
Gln Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Glu Gly Leu His Asp
            180                 185                 190
Val His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe
        195                 200                 205
Thr Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys
    210                 215                 220
Pro Lys Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser
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225                 230                 235                 240
Cys Glu Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala
                245                 250                 255
Asn Ala Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Asp Asp Ala
            260                 265                 270
Phe Val Cys Gly Ala Ile Ala Val Ala Gln Ser Ile Arg Met Ser Gly
        275                 280                 285
Ser Thr Arg Asn Leu Val Ile Leu Val Asp Asp Ser Ile Ser Glu Tyr
    290                 295                 300
His Arg Ser Gly Leu Glu Ser Ala Gly Trp Lys Ile His Thr Phe Gln
305                 310                 315                 320
Arg Ile Arg Asn Pro Lys Ala Glu Ala Asn Ala Tyr Asn Gln Trp Asn
                325                 330                 335
Tyr Ser Lys Phe Arg Leu Trp Glu Leu Thr Glu Tyr Asn Lys Ile Ile
            340                 345                 350
Phe Ile Asp Ala Asp Met Leu Ile Leu Arg Asn Met Asp Phe Leu Phe
        355                 360                 365
Glu Tyr Pro Glu Ile Ser Thr Thr Gly Asn Asp Gly Thr Leu Phe Asn
    370                 375                 380
Ser Gly Leu Met Val Ile Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu
385                 390                 395                 400
Met Asp His Ile Asn Asp Ile Asn Ser Tyr Asn Gly Gly Asp Gln Gly
                405                 410                 415
Tyr Leu Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met
            420                 425                 430
Asn Phe Leu Lys His Phe Trp Glu Gly Asp Thr Pro Lys His Arg Lys
        435                 440                 445
Ser Lys Thr Arg Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu
    450                 455                 460
His Tyr Leu Gly Tyr Asn Lys Pro Trp Val Cys Phe Arg Asp Tyr Asp
465                 470                 475                 480
Cys Asn Trp Asn Val Val Gly Tyr His Gln Phe Ala Ser Asp Glu Ala
                485                 490                 495
His Lys Thr Trp Trp Arg Val His Asp Ala Met Pro Lys Lys Leu Gln
            500                 505                 510
Arg Phe Cys Leu Leu Ser Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp
        515                 520                 525
Arg Arg Gln Ala Glu Lys Ala Asn Tyr Arg Asp Gly His Trp Arg Ile
    530                 535                 540
Lys Ile Lys Asp Lys Arg Leu Thr Thr Cys Phe Glu Asp Phe Cys Phe
545                 550                 555                 560
Trp Glu Ser Met Leu Trp His Trp Gly Asp Tyr Glu Ile Leu Glu Thr
                565                 570                 575
Asp Pro Gly Leu Thr Glu Thr Met Ile Pro Ser Ser Ser Pro Met Glu
            580                 585                 590
Ser Arg His Arg Leu Ser Phe Ser Asn Glu Lys Thr Ser Arg Arg Arg
        595                 600                 605
Phe Gln Arg Ile Glu Lys Gly Val Lys Phe Asn Thr Leu Lys Leu Val
    610                 615                 620
Leu Ile Cys Ile Met Leu Gly Ala Leu Phe Thr Ile Tyr Arg Phe Arg
625                 630                 635                 640
Tyr Pro Pro Leu Gln Ile Pro Glu Ile Pro Thr Ser Phe Gly Leu Thr
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645 650 655
Thr Asp Pro Arg Tyr Val Ala Thr Ala Glu Ile Asn Trp Asn His Met
660 665 670
Ser Asn Leu Val Glu Lys His Val Phe Gly Arg Ser Glu Tyr Gln Gly
675 680 685
Ile Gly Leu Ile Asn Leu Asn Asp Asn Glu Ile Asp Arg Phe Lys Glu
690 695 700
Val Thr Lys Ser Asp Cys Asp His Val Ala Leu His Leu Asp Tyr Ala
705 710 715 720
Ala Lys Asn Ile Thr Trp Glu Ser Leu Tyr Pro Glu Trp Ile Asp Glu
725 730 735
Val Glu Glu Phe Glu Val Pro Thr Cys Pro Ser Leu Pro Leu Ile Gln
740 745 750
Ile Pro Gly Lys Pro Arg Ile Asp Leu Val Ile Ala Lys Leu Pro Cys
755 760 765
Asp Lys Ser Gly Lys Trp Ser Arg Asp Val Ala Arg Leu His Leu Gln
770 775 780
Leu Ala Ala Ala Arg Val Ala Ala Ser Ser Lys Gly Leu His Asn Val
785 790 795 800
His Val Ile Leu Val Ser Asp Cys Phe Pro Ile Pro Asn Leu Phe Thr
805 810 815
Gly Gln Glu Leu Val Ala Arg Gln Gly Asn Ile Trp Leu Tyr Lys Pro
820 825 830
Asn Leu His Gln Leu Arg Gln Lys Leu Gln Leu Pro Val Gly Ser Cys
835 840 845
Glu Leu Ser Val Pro Leu Gln Ala Lys Asp Asn Phe Tyr Ser Ala Gly
850 855 860
Ala Lys Lys Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Gln Phe Tyr
865 870 875 880
Val Cys Gly Ala Ile Ala Ala Ala Gln Ser Ile Arg Met Ser Gly Ser
885 890 895
Thr Arg Asp Leu Val Ile Leu Val Asp Glu Thr Ile Ser Glu Tyr His
900 905 910
Lys Ser Gly Leu Val Ala Ala Gly Trp Lys Ile Gln Met Phe Gln Arg
915 920 925
Ile Arg Asn Pro Asn Ala Val Pro Asn Ala Tyr Asn Glu Trp Asn Tyr
930 935 940
Ser Lys Phe Arg Leu Trp Gln Leu Thr Glu Tyr Ser Lys Ile Ile Phe
945 950 955 960
Ile Asp Ala Asp Met Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Glu
965 970 975
Phe Pro Glu Ile Ser Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser
980 985 990
Gly Leu Met Val Val Glu Pro Ser Asn Ser Thr Phe Gln Leu Leu Met
995 1000 1005
Asp Asn Ile Asn Glu Val Val Ser Tyr Asn Gly Gly Asp Gln Gly Tyr
1010 1015 1020
Leu Asn Glu Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn
1025 1030 1035 1040
Phe Leu Lys His Phe Trp Glu Gly Asp Glu Pro Glu Ile Lys Lys Met
1045 1050 1055
Lys Thr Ser Leu Phe Gly Ala Asp Pro Pro Ile Leu Tyr Val Leu His

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          1060                1065                1070
Tyr Leu Gly Tyr Asn Lys Pro Trp Leu Cys Phe Arg Asp Tyr Asp Cys
       1075                1080                1085
Asn Trp Asn Val Asp Ile Phe Gln Glu Phe Ala Ser Asp Glu Ala His
   1090                1095                1100
Lys Thr Trp Trp Arg Val His Asp Ala Met Pro Glu Asn Leu His Lys
1105                1110                1115                1120
Phe Cys Leu Leu Arg Ser Lys Gln Lys Ala Gln Leu Glu Trp Asp Arg
               1125                1130                1135
Arg Gln Ala Glu Lys Gly Asn Tyr Lys Asp Gly His Trp Lys Ile Lys
           1140                1145                1150
Ile Lys Asp Lys Arg Leu Lys Thr Cys Phe Glu Asp Phe Cys Phe Trp
       1155                1160                1165
Glu Ser Met Leu Trp His Trp Gly Glu Thr Asn Ser Thr Asn Asn Ser
   1170                1175                1180
Ser Thr Thr Thr Thr Ser Ser Pro Pro His Lys Thr Ala Leu Pro Ser
1185                1190                1195                1200
Leu
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<222> (1)..(1653)

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Met Gly Ala Lys Ser Lys Ser Ser Ser Thr Arg Phe Phe Met Phe Tyr
  1               5                  10                  15

ctt ata cta ata tca ttg tcg ttt ttg ggt ttg ctc tta aac ttt aaa   96
Leu Ile Leu Ile Ser Leu Ser Phe Leu Gly Leu Leu Leu Asn Phe Lys
             20                  25                  30

cct ctg ttt ctg ctc aac ccc atg atc gct tct cct tcg ata gtt gag   144
Pro Leu Phe Leu Leu Asn Pro Met Ile Ala Ser Pro Ser Ile Val Glu
         35                  40                  45

att cgt tat tct ttg ccg gaa ccg gtt aaa cgg act ccg ata tgg ctc   192
Ile Arg Tyr Ser Leu Pro Glu Pro Val Lys Arg Thr Pro Ile Trp Leu
     50                  55                  60

cga ctc att aga aac tat ctt ccg gat gag aaa aag atc cga gtg ggt   240
Arg Leu Ile Arg Asn Tyr Leu Pro Asp Glu Lys Lys Ile Arg Val Gly
 65                  70                  75                  80

ctt ctc aac atc gca gag aac gag cga gag agc tac gag gca agc ggg   288
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Leu Leu Asn Ile Ala Glu Asn Glu Arg Glu Ser Tyr Glu Ala Ser Gly
                  85                  90                  95

acg tcg atc ttg gag aat gtc cac gtg tcg ctc gat cct ctt ccg aac   336
Thr Ser Ile Leu Glu Asn Val His Val Ser Leu Asp Pro Leu Pro Asn
            100                 105                 110

aat ctg aca tgg acg agt tta ttc ccg gtt tgg atc gac gag gat cac   384
Asn Leu Thr Trp Thr Ser Leu Phe Pro Val Trp Ile Asp Glu Asp His
        115                 120                 125

acg tgg cac att cct agt tgt cca gaa gtc cct ctc cct aag atg gaa   432
Thr Trp His Ile Pro Ser Cys Pro Glu Val Pro Leu Pro Lys Met Glu
    130                 135                 140

ggt tcc gaa gct gac gtg gac gtc gtc gtt gtc aaa gtc ccg tgc gat   480
Gly Ser Glu Ala Asp Val Asp Val Val Val Val Lys Val Pro Cys Asp
145                 150                 155                 160

ggt ttc tcg gag aag aga ggg tta aga gac gtt ttc agg cta cag gtg   528
Gly Phe Ser Glu Lys Arg Gly Leu Arg Asp Val Phe Arg Leu Gln Val
                165                 170                 175

aat ctg gcg gca gcg aat ctt gtg gtg gag agt ggt cgg agg aat gtt   576
Asn Leu Ala Ala Ala Asn Leu Val Val Glu Ser Gly Arg Arg Asn Val
            180                 185                 190

gat cgg act gtg tac gtt gtc ttc atc gga tct tgt ggg cct atg cat   624
Asp Arg Thr Val Tyr Val Val Phe Ile Gly Ser Cys Gly Pro Met His
        195                 200                 205

gag atc ttt agg tgt gat gag cgc gtg aag cgc gtg ggg gac tat tgg   672
Glu Ile Phe Arg Cys Asp Glu Arg Val Lys Arg Val Gly Asp Tyr Trp
    210                 215                 220

gtc tat agg cct gat ctt acg agg ttg aag cag aag ctt ctc atg cct   720
Val Tyr Arg Pro Asp Leu Thr Arg Leu Lys Gln Lys Leu Leu Met Pro
225                 230                 235                 240

cct ggt tca tgt cag att gct ccg cta ggt caa gga gaa gca tgg ata   768
Pro Gly Ser Cys Gln Ile Ala Pro Leu Gly Gln Gly Glu Ala Trp Ile
                245                 250                 255

caa gac aag aac aga aat ctc aca tcc gaa aaa act aca tta tca tca   816
Gln Asp Lys Asn Arg Asn Leu Thr Ser Glu Lys Thr Thr Leu Ser Ser
            260                 265                 270

ttt act gcc caa cgt gtc gct tac gtg acg tta cta cac tca tcg gag   864
Phe Thr Ala Gln Arg Val Ala Tyr Val Thr Leu Leu His Ser Ser Glu
        275                 280                 285

gta tac gta tgc gga gca ata gcc tta gca caa agc ata agg caa tct   912
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Val Tyr Val Cys Gly Ala Ile Ala Leu Ala Gln Ser Ile Arg Gln Ser
    290                 295                 300

gga tca acc aag gac atg att ctc ctc cac gat gac tct ata acc aac   960
Gly Ser Thr Lys Asp Met Ile Leu Leu His Asp Asp Ser Ile Thr Asn
305                 310                 315                 320

atc tct ctc att ggc cta agc ctt gct ggc tgg aaa cta cgg cga gtg   1008
Ile Ser Leu Ile Gly Leu Ser Leu Ala Gly Trp Lys Leu Arg Arg Val
                325                 330                 335

gag aga att cgt agt cct ttt tcc aag aag cgt tct tac aat gag tgg   1056
Glu Arg Ile Arg Ser Pro Phe Ser Lys Lys Arg Ser Tyr Asn Glu Trp
            340                 345                 350

aac tac agt aag tta cgt gtg tgg caa gtg aca gat tac gac aaa cta   1104
Asn Tyr Ser Lys Leu Arg Val Trp Gln Val Thr Asp Tyr Asp Lys Leu
        355                 360                 365

gtg ttt ata gac gca gac ttc atc atc gtc aag aat att gat tac ctt   1152
Val Phe Ile Asp Ala Asp Phe Ile Ile Val Lys Asn Ile Asp Tyr Leu
    370                 375                 380

ttc tcc tat cct caa ctt tct gcc gct ggc aat aac aaa gtc ttg ttc   1200
Phe Ser Tyr Pro Gln Leu Ser Ala Ala Gly Asn Asn Lys Val Leu Phe
385                 390                 395                 400

aac tca gga gtc atg gtt ctg gag cca tca gct tgt tta ttc gag gat   1248
Asn Ser Gly Val Met Val Leu Glu Pro Ser Ala Cys Leu Phe Glu Asp
                405                 410                 415

ttg atg ctt aaa tca ttc aag atc ggg tca tac aac ggg gga gac caa   1296
Leu Met Leu Lys Ser Phe Lys Ile Gly Ser Tyr Asn Gly Gly Asp Gln
            420                 425                 430

gga ttt ctg aac gaa tat ttc gtg tgg tgg cat agg cat gat aaa gcg   1344
Gly Phe Leu Asn Glu Tyr Phe Val Trp Trp His Arg His Asp Lys Ala
        435                 440                 445

cgc aat ctt cca gaa aat tta gag ggc ata cac tac ttg gga cta aaa   1392
Arg Asn Leu Pro Glu Asn Leu Glu Gly Ile His Tyr Leu Gly Leu Lys
    450                 455                 460

cca tgg cga tgt tac aga gac tac gat tgt aac tgg gac ttg aaa acg   1440
Pro Trp Arg Cys Tyr Arg Asp Tyr Asp Cys Asn Trp Asp Leu Lys Thr
465                 470                 475                 480

cga cgt gtg tat gca agc gag tcg gtg cat gcg aga tgg tgg aaa gtg   1488
Arg Arg Val Tyr Ala Ser Glu Ser Val His Ala Arg Trp Trp Lys Val
                485                 490                 495

tac gac aag atg cct aag aag ctg aaa ggt tat tgt ggt ttg aat ctt   1536
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Tyr Asp Lys Met Pro Lys Lys Leu Lys Gly Tyr Cys Gly Leu Asn Leu
            500                 505                 510

aag atg gag aag aac gtt gag aag tgg agg aaa atg gct aag ctc aat   1584
Lys Met Glu Lys Asn Val Glu Lys Trp Arg Lys Met Ala Lys Leu Asn
        515                 520                 525

ggt ttt cct gaa aat cat tgg aaa att aga ata aaa gat cct agg aag   1632
Gly Phe Pro Glu Asn His Trp Lys Ile Arg Ile Lys Asp Pro Arg Lys
    530                 535                 540

aag aac cgt cta agt caa tga                                      1653
Lys Asn Arg Leu Ser Gln
545                 550
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<210> 9
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<213> Arabidopsis thaliana

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Met Gly Ala Lys Ser Lys Ser Ser Ser Thr Arg Phe Phe Met Phe Tyr
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Leu Ile Leu Ile Ser Leu Ser Phe Leu Gly Leu Leu Leu Asn Phe Lys
             20                  25                  30
Pro Leu Phe Leu Leu Asn Pro Met Ile Ala Ser Pro Ser Ile Val Glu
         35                  40                  45
Ile Arg Tyr Ser Leu Pro Glu Pro Val Lys Arg Thr Pro Ile Trp Leu
     50                  55                  60
Arg Leu Ile Arg Asn Tyr Leu Pro Asp Glu Lys Lys Ile Arg Val Gly
 65                  70                  75                  80
Leu Leu Asn Ile Ala Glu Asn Glu Arg Glu Ser Tyr Glu Ala Ser Gly
                 85                  90                  95
Thr Ser Ile Leu Glu Asn Val His Val Ser Leu Asp Pro Leu Pro Asn
            100                 105                 110
Asn Leu Thr Trp Thr Ser Leu Phe Pro Val Trp Ile Asp Glu Asp His
        115                 120                 125
Thr Trp His Ile Pro Ser Cys Pro Glu Val Pro Leu Pro Lys Met Glu
    130                 135                 140
Gly Ser Glu Ala Asp Val Asp Val Val Val Val Lys Val Pro Cys Asp
145                 150                 155                 160
Gly Phe Ser Glu Lys Arg Gly Leu Arg Asp Val Phe Arg Leu Gln Val
                165                 170                 175
Asn Leu Ala Ala Ala Asn Leu Val Val Glu Ser Gly Arg Arg Asn Val
            180                 185                 190
Asp Arg Thr Val Tyr Val Val Phe Ile Gly Ser Cys Gly Pro Met His
        195                 200                 205
Glu Ile Phe Arg Cys Asp Glu Arg Val Lys Arg Val Gly Asp Tyr Trp
    210                 215                 220
Val Tyr Arg Pro Asp Leu Thr Arg Leu Lys Gln Lys Leu Leu Met Pro
225                 230                 235                 240
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Pro Gly Ser Cys Gln Ile Ala Pro Leu Gly Gln Gly Glu Ala Trp Ile
                245                 250                 255
Gln Asp Lys Asn Arg Asn Leu Thr Ser Glu Lys Thr Thr Leu Ser Ser
            260                 265                 270
Phe Thr Ala Gln Arg Val Ala Tyr Val Thr Leu Leu His Ser Ser Glu
        275                 280                 285
Val Tyr Val Cys Gly Ala Ile Ala Leu Ala Gln Ser Ile Arg Gln Ser
    290                 295                 300
Gly Ser Thr Lys Asp Met Ile Leu Leu His Asp Asp Ser Ile Thr Asn
305                 310                 315                 320
Ile Ser Leu Ile Gly Leu Ser Leu Ala Gly Trp Lys Leu Arg Arg Val
                325                 330                 335
Glu Arg Ile Arg Ser Pro Phe Ser Lys Lys Arg Ser Tyr Asn Glu Trp
            340                 345                 350
Asn Tyr Ser Lys Leu Arg Val Trp Gln Val Thr Asp Tyr Asp Lys Leu
        355                 360                 365
Val Phe Ile Asp Ala Asp Phe Ile Ile Val Lys Asn Ile Asp Tyr Leu
    370                 375                 380
Phe Ser Tyr Pro Gln Leu Ser Ala Ala Gly Asn Asn Lys Val Leu Phe
385                 390                 395                 400
Asn Ser Gly Val Met Val Leu Glu Pro Ser Ala Cys Leu Phe Glu Asp
                405                 410                 415
Leu Met Leu Lys Ser Phe Lys Ile Gly Ser Tyr Asn Gly Gly Asp Gln
            420                 425                 430
Gly Phe Leu Asn Glu Tyr Phe Val Trp Trp His Arg His Asp Lys Ala
        435                 440                 445
Arg Asn Leu Pro Glu Asn Leu Glu Gly Ile His Tyr Leu Gly Leu Lys
    450                 455                 460
Pro Trp Arg Cys Tyr Arg Asp Tyr Asp Cys Asn Trp Asp Leu Lys Thr
465                 470                 475                 480
Arg Arg Val Tyr Ala Ser Glu Ser Val His Ala Arg Trp Trp Lys Val
                485                 490                 495
Tyr Asp Lys Met Pro Lys Lys Leu Lys Gly Tyr Cys Gly Leu Asn Leu
            500                 505                 510
Lys Met Glu Lys Asn Val Glu Lys Trp Arg Lys Met Ala Lys Leu Asn
        515                 520                 525
Gly Phe Pro Glu Asn His Trp Lys Ile Arg Ile Lys Asp Pro Arg Lys
    530                 535                 540
Lys Asn Arg Leu Ser Gln
545                 550
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Met Gly Thr Lys Thr His Asn Ser Arg Gly Lys Ile Phe Met Ile Tyr
  1               5                  10                  15

cta atc cta gtc tca ttg tca ctt cta ggt ttg atc tta cct ttt aaa   96
Leu Ile Leu Val Ser Leu Ser Leu Leu Gly Leu Ile Leu Pro Phe Lys
            20                  25                  30

cct ctt ttc cgg att act tct cca tct tca acg tta cgg att gat ctt  144
Pro Leu Phe Arg Ile Thr Ser Pro Ser Ser Thr Leu Arg Ile Asp Leu
         35                  40                  45

cca tcg ccg caa gtc aac aaa aac ccg aaa tgg ctt cga ctc atc cgt  192
Pro Ser Pro Gln Val Asn Lys Asn Pro Lys Trp Leu Arg Leu Ile Arg
     50                  55                  60

aac tat cta cca gag aaa aga atc caa gtc ggc ttc ctt aac ata gac  240
Asn Tyr Leu Pro Glu Lys Arg Ile Gln Val Gly Phe Leu Asn Ile Asp
 65                  70                  75                  80

gag aaa gag cgt gag agc tac gag gct cgt gga ccg ttg gta ctt aag  288
Glu Lys Glu Arg Glu Ser Tyr Glu Ala Arg Gly Pro Leu Val Leu Lys
                85                  90                  95

aac atc cac gtg ccg ctt gat cat ata ccc aag aat gtc act tgg aag  336
Asn Ile His Val Pro Leu Asp His Ile Pro Lys Asn Val Thr Trp Lys
            100                 105                 110

agt ctt tac ccg gag tgg atc aac gag gaa gct tct acc tgt ccg gag  384
Ser Leu Tyr Pro Glu Trp Ile Asn Glu Glu Ala Ser Thr Cys Pro Glu
        115                 120                 125

atc cct ctc cct cag cca gaa ggt tct gat gct aac gtg gac gtt att  432
Ile Pro Leu Pro Gln Pro Glu Gly Ser Asp Ala Asn Val Asp Val Ile
    130                 135                 140

gtt gct aga gtt cca tgt gat ggt tgg tcg gcg aat aaa ggg ctt agg  480
Val Ala Arg Val Pro Cys Asp Gly Trp Ser Ala Asn Lys Gly Leu Arg
145                 150                 155                 160

gac gtt ttt agg ctt cag gtt aat ttg gcc gca gcg aat cta gcc gtc  528
Asp Val Phe Arg Leu Gln Val Asn Leu Ala Ala Ala Asn Leu Ala Val
                165                 170                 175

caa agt ggg ttg agg acg gtt aat cag gcg gtc tac gtt gta ttc atc  576
Gln Ser Gly Leu Arg Thr Val Asn Gln Ala Val Tyr Val Val Phe Ile
            180                 185                 190

ggc tca tgt ggg cct atg cat gag att ttc ccg tgc gat gag cgc gtg  624
Gly Ser Cys Gly Pro Met His Glu Ile Phe Pro Cys Asp Glu Arg Val
        195                 200                 205
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atg cgc gtg gag gat tat tgg gtg tat aag cct tat ctc cca agg ttg   672
Met Arg Val Glu Asp Tyr Trp Val Tyr Lys Pro Tyr Leu Pro Arg Leu
    210                 215                 220

aag cag aag ctt ctc atg cct gtt ggt tca tgt cag att gct cct tca   720
Lys Gln Lys Leu Leu Met Pro Val Gly Ser Cys Gln Ile Ala Pro Ser
225                 230                 235                 240

ttt gct caa ttt ggt caa gaa gca tgg aga cca aaa cat gaa gat aat   768
Phe Ala Gln Phe Gly Gln Glu Ala Trp Arg Pro Lys His Glu Asp Asn
                245                 250                 255

ctt gca tca aag gca gtc aca gcc tta ccc cgt cgc tta cgg gtt gcc   816
Leu Ala Ser Lys Ala Val Thr Ala Leu Pro Arg Arg Leu Arg Val Ala
            260                 265                 270

tac gtg aca gta cta cac tcg tca gaa gcc tat gtt tgt ggg gca ata   864
Tyr Val Thr Val Leu His Ser Ser Glu Ala Tyr Val Cys Gly Ala Ile
        275                 280                 285

gct tta gcg caa agt ata aga caa tca gga tcg cat aag gac atg att   912
Ala Leu Ala Gln Ser Ile Arg Gln Ser Gly Ser His Lys Asp Met Ile
    290                 295                 300

ctc ctc cat gat cat acc ata acc aac aag tct ctt att ggt ctc agc   960
Leu Leu His Asp His Thr Ile Thr Asn Lys Ser Leu Ile Gly Leu Ser
305                 310                 315                 320

gct gcg gga tgg aat ctc cgg cta atc gac agg atc cgc agt cct ttt   1008
Ala Ala Gly Trp Asn Leu Arg Leu Ile Asp Arg Ile Arg Ser Pro Phe
                325                 330                 335

tcg caa aaa gac tct tat aat gag tgg aac tat agc aaa tta cgt gtg   1056
Ser Gln Lys Asp Ser Tyr Asn Glu Trp Asn Tyr Ser Lys Leu Arg Val
            340                 345                 350

tgg caa gta act gac tac gat aaa ctt gtg ttc ata gac gca gat ttc   1104
Trp Gln Val Thr Asp Tyr Asp Lys Leu Val Phe Ile Asp Ala Asp Phe
        355                 360                 365

atc atc ctc aag aaa ctt gat cat ctc ttc tac tat cca caa ctc tca   1152
Ile Ile Leu Lys Lys Leu Asp His Leu Phe Tyr Tyr Pro Gln Leu Ser
    370                 375                 380

gct tca ggc aac gac aaa gtg tta ttc aac tcc gga atc atg gtt ctc   1200
Ala Ser Gly Asn Asp Lys Val Leu Phe Asn Ser Gly Ile Met Val Leu
385                 390                 395                 400

gag cca tcg gca tgt atg ttt aaa gat tta atg gag aaa tcg ttc aag   1248
Glu Pro Ser Ala Cys Met Phe Lys Asp Leu Met Glu Lys Ser Phe Lys
                405                 410                 415
```

```
att gag tca tac aac gga gga gac caa gga ttc ctt aat gag ata ttt   1296
Ile Glu Ser Tyr Asn Gly Gly Asp Gln Gly Phe Leu Asn Glu Ile Phe
            420                 425                 430

gta tgg tgg cac agg tta tcg aaa cga gtg aac aca atg aag tac ttc   1344
Val Trp Trp His Arg Leu Ser Lys Arg Val Asn Thr Met Lys Tyr Phe
        435                 440                 445

gac gaa aaa aat cat cga aga cac gat ctt cct gag aat gta gaa ggt   1392
Asp Glu Lys Asn His Arg Arg His Asp Leu Pro Glu Asn Val Glu Gly
    450                 455                 460

ctg cac tac ttg ggg ttg aaa cca tgg gta tgt tat aga gac tat gat   1440
Leu His Tyr Leu Gly Leu Lys Pro Trp Val Cys Tyr Arg Asp Tyr Asp
465                 470                 475                 480

tgc aat tgg gac att agc gaa cga cgc gtg ttt gca agc gat tct gtg   1488
Cys Asn Trp Asp Ile Ser Glu Arg Arg Val Phe Ala Ser Asp Ser Val
                485                 490                 495

cac gaa aaa tgg tgg aaa gtg tat gac aaa atg tca gag cag ttg aaa   1536
His Glu Lys Trp Trp Lys Val Tyr Asp Lys Met Ser Glu Gln Leu Lys
            500                 505                 510

ggt tat tgt ggt ttg aat aag aat atg gag aag agg att gag aag tgg   1584
Gly Tyr Cys Gly Leu Asn Lys Asn Met Glu Lys Arg Ile Glu Lys Trp
        515                 520                 525

aga aga atc gct aag aac aat agt ttg cct gat agg cat tgg gag att   1632
Arg Arg Ile Ala Lys Asn Asn Ser Leu Pro Asp Arg His Trp Glu Ile
    530                 535                 540

gaa gtg aga gat cct agg aag acg aat ctt ctt gtt cag tga           1674
Glu Val Arg Asp Pro Arg Lys Thr Asn Leu Leu Val Gln
545                 550                 555
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Leu Ile Leu Val Ser Leu Ser Leu Leu Gly Leu Ile Leu Pro Phe Lys
             20                  25                  30
Pro Leu Phe Arg Ile Thr Ser Pro Ser Ser Thr Leu Arg Ile Asp Leu
         35                  40                  45
Pro Ser Pro Gln Val Asn Lys Asn Pro Lys Trp Leu Arg Leu Ile Arg
     50                  55                  60
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```
Asn Tyr Leu Pro Glu Lys Arg Ile Gln Val Gly Phe Leu Asn Ile Asp
 65                  70                  75                  80
Glu Lys Glu Arg Glu Ser Tyr Glu Ala Arg Gly Pro Leu Val Leu Lys
                85                  90                  95
Asn Ile His Val Pro Leu Asp His Ile Pro Lys Asn Val Thr Trp Lys
            100                 105                 110
Ser Leu Tyr Pro Glu Trp Ile Asn Glu Glu Ala Ser Thr Cys Pro Glu
        115                 120                 125
Ile Pro Leu Pro Gln Pro Glu Gly Ser Asp Ala Asn Val Asp Val Ile
    130                 135                 140
Val Ala Arg Val Pro Cys Asp Gly Trp Ser Ala Asn Lys Gly Leu Arg
145                 150                 155                 160
Asp Val Phe Arg Leu Gln Val Asn Leu Ala Ala Ala Asn Leu Ala Val
                165                 170                 175
Gln Ser Gly Leu Arg Thr Val Asn Gln Ala Val Tyr Val Val Phe Ile
            180                 185                 190
Gly Ser Cys Gly Pro Met His Glu Ile Phe Pro Cys Asp Glu Arg Val
        195                 200                 205
Met Arg Val Glu Asp Tyr Trp Val Tyr Lys Pro Tyr Leu Pro Arg Leu
    210                 215                 220
Lys Gln Lys Leu Leu Met Pro Val Gly Ser Cys Gln Ile Ala Pro Ser
225                 230                 235                 240
Phe Ala Gln Phe Gly Gln Glu Ala Trp Arg Pro Lys His Glu Asp Asn
                245                 250                 255
Leu Ala Ser Lys Ala Val Thr Ala Leu Pro Arg Arg Leu Arg Val Ala
            260                 265                 270
Tyr Val Thr Val Leu His Ser Ser Glu Ala Tyr Val Cys Gly Ala Ile
        275                 280                 285
Ala Leu Ala Gln Ser Ile Arg Gln Ser Gly Ser His Lys Asp Met Ile
    290                 295                 300
Leu Leu His Asp His Thr Ile Thr Asn Lys Ser Leu Ile Gly Leu Ser
305                 310                 315                 320
Ala Ala Gly Trp Asn Leu Arg Leu Ile Asp Arg Ile Arg Ser Pro Phe
                325                 330                 335
Ser Gln Lys Asp Ser Tyr Asn Glu Trp Asn Tyr Ser Lys Leu Arg Val
            340                 345                 350
Trp Gln Val Thr Asp Tyr Asp Lys Leu Val Phe Ile Asp Ala Asp Phe
        355                 360                 365
Ile Ile Leu Lys Lys Leu Asp His Leu Phe Tyr Tyr Pro Gln Leu Ser
    370                 375                 380
Ala Ser Gly Asn Asp Lys Val Leu Phe Asn Ser Gly Ile Met Val Leu
385                 390                 395                 400
Glu Pro Ser Ala Cys Met Phe Lys Asp Leu Met Glu Lys Ser Phe Lys
                405                 410                 415
Ile Glu Ser Tyr Asn Gly Gly Asp Gln Gly Phe Leu Asn Glu Ile Phe
            420                 425                 430
Val Trp Trp His Arg Leu Ser Lys Arg Val Asn Thr Met Lys Tyr Phe
        435                 440                 445
Asp Glu Lys Asn His Arg Arg His Asp Leu Pro Glu Asn Val Glu Gly
    450                 455                 460
Leu His Tyr Leu Gly Leu Lys Pro Trp Val Cys Tyr Arg Asp Tyr Asp
465                 470                 475                 480
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```
Cys Asn Trp Asp Ile Ser Glu Arg Arg Val Phe Ala Ser Asp Ser Val
                485                 490                 495
His Glu Lys Trp Trp Lys Val Tyr Asp Lys Met Ser Glu Gln Leu Lys
            500                 505                 510
Gly Tyr Cys Gly Leu Asn Lys Asn Met Glu Lys Arg Ile Glu Lys Trp
        515                 520                 525
Arg Arg Ile Ala Lys Asn Asn Ser Leu Pro Asp Arg His Trp Glu Ile
    530                 535                 540
Glu Val Arg Asp Pro Arg Lys Thr Asn Leu Leu Val Gln
545                 550                 555
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<220>
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<222> (1)..(1002)

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  1               5                  10                  15

gca ggt gta gac aaa gtg tat gac cta acg aag ata gaa gca gag aca   96
Ala Gly Val Asp Lys Val Tyr Asp Leu Thr Lys Ile Glu Ala Glu Thr
             20                  25                  30

aaa cga cca aaa cgt gaa gcc tac gta act gtt ctt cac tct tcc gag   144
Lys Arg Pro Lys Arg Glu Ala Tyr Val Thr Val Leu His Ser Ser Glu
         35                  40                  45

tct tat gtc tgt ggt gcc ata act ttg gct caa agc ctc ctt cag aca   192
Ser Tyr Val Cys Gly Ala Ile Thr Leu Ala Gln Ser Leu Leu Gln Thr
     50                  55                  60

aac acc aaa cgc gat ctt atc ctt ctc cac gat gac tcc atc tcc att   240
Asn Thr Lys Arg Asp Leu Ile Leu Leu His Asp Asp Ser Ile Ser Ile
 65                  70                  75                  80

acc aaa ctt cga gct ctc gcc gcc gca gga tgg aag ctt cgt cgg atc   288
Thr Lys Leu Arg Ala Leu Ala Ala Ala Gly Trp Lys Leu Arg Arg Ile
                 85                  90                  95

att cga atc aga aac cca ctt gcg gag aag gac tcg tac aat gaa tac   336
Ile Arg Ile Arg Asn Pro Leu Ala Glu Lys Asp Ser Tyr Asn Glu Tyr
            100                 105                 110

aac tac agc aag ttt cga ctc tgg caa ttg aca gat tac gac aaa gtg   384
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Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val
        115                 120                 125

atc ttc att gat gcc gac atc atc gtc tta cgt aac ctt gat ctt ctc   432
Ile Phe Ile Asp Ala Asp Ile Ile Val Leu Arg Asn Leu Asp Leu Leu
    130                 135                 140

ttc cat ttt cct cag atg tcg gcc acc gga aat gat gta tgg ata tat   480
Phe His Phe Pro Gln Met Ser Ala Thr Gly Asn Asp Val Trp Ile Tyr
145                 150                 155                 160

aat tca ggc atc atg gtc atc gag cct tct aat tgt acg ttt act aca   528
Asn Ser Gly Ile Met Val Ile Glu Pro Ser Asn Cys Thr Phe Thr Thr
                165                 170                 175

atc atg agc cag cga agc gag atc gtt tca tac aac ggt gga gat caa   576
Ile Met Ser Gln Arg Ser Glu Ile Val Ser Tyr Asn Gly Gly Asp Gln
            180                 185                 190

ggg tac cta aac gag ata ttt gtg tgg tgg cac cga ttg cct cga cga   624
Gly Tyr Leu Asn Glu Ile Phe Val Trp Trp His Arg Leu Pro Arg Arg
        195                 200                 205

gta aac ttt ctg aag aac ttc tgg tcg aac aca acc aaa gaa aga aac   672
Val Asn Phe Leu Lys Asn Phe Trp Ser Asn Thr Thr Lys Glu Arg Asn
    210                 215                 220

atc aag aac aac ctc ttc gcc gcg gag ccg cct cag gtc tac gcg gtc   720
Ile Lys Asn Asn Leu Phe Ala Ala Glu Pro Pro Gln Val Tyr Ala Val
225                 230                 235                 240

cac tac tta ggt tgg aaa cca tgg ctt tgc tat agg gac tac gat tgc   768
His Tyr Leu Gly Trp Lys Pro Trp Leu Cys Tyr Arg Asp Tyr Asp Cys
                245                 250                 255

aac tac gac gtg gac gag cag ttg gtg tac gct agt gat gcg gct cac   816
Asn Tyr Asp Val Asp Glu Gln Leu Val Tyr Ala Ser Asp Ala Ala His
            260                 265                 270

gtt agg tgg tgg aaa gtg cac gac tcc atg gac gat gca ttg caa aag   864
Val Arg Trp Trp Lys Val His Asp Ser Met Asp Asp Ala Leu Gln Lys
        275                 280                 285

ttt tgc agg ctg acg aaa aag agg aga acg gag atc aac tgg gag agg   912
Phe Cys Arg Leu Thr Lys Lys Arg Arg Thr Glu Ile Asn Trp Glu Arg
    290                 295                 300

agg aaa gca agg ctt aga ggt tcc act gat tat cat tgg aag atc aat   960
Arg Lys Ala Arg Leu Arg Gly Ser Thr Asp Tyr His Trp Lys Ile Asn
305                 310                 315                 320

gtc act gat cca aga cga cgt cgt tct tat ttg att ggt taa          1002
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Val Thr Asp Pro Arg Arg Arg Arg Ser Tyr Leu Ile Gly
325 330

<210> 13
<211> 333
<212> PRT
<213> Arabidopsis thaliana

<400> 13
Met Ala Leu Leu Asn Glu Leu Met Ser Phe Phe Ile Gln Lys Gln Lys
1 5 10 15
Ala Gly Val Asp Lys Val Tyr Asp Leu Thr Lys Ile Glu Ala Glu Thr
20 25 30
Lys Arg Pro Lys Arg Glu Ala Tyr Val Thr Val Leu His Ser Ser Glu
35 40 45
Ser Tyr Val Cys Gly Ala Ile Thr Leu Ala Gln Ser Leu Leu Gln Thr
50 55 60
Asn Thr Lys Arg Asp Leu Ile Leu Leu His Asp Asp Ser Ile Ser Ile
65 70 75 80
Thr Lys Leu Arg Ala Leu Ala Ala Ala Gly Trp Lys Leu Arg Arg Ile
85 90 95
Ile Arg Ile Arg Asn Pro Leu Ala Glu Lys Asp Ser Tyr Asn Glu Tyr
100 105 110
Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val
115 120 125
Ile Phe Ile Asp Ala Asp Ile Ile Val Leu Arg Asn Leu Asp Leu Leu
130 135 140
Phe His Phe Pro Gln Met Ser Ala Thr Gly Asn Asp Val Trp Ile Tyr
145 150 155 160
Asn Ser Gly Ile Met Val Ile Glu Pro Ser Asn Cys Thr Phe Thr Thr
165 170 175
Ile Met Ser Gln Arg Ser Glu Ile Val Ser Tyr Asn Gly Gly Asp Gln
180 185 190
Gly Tyr Leu Asn Glu Ile Phe Val Trp Trp His Arg Leu Pro Arg Arg
195 200 205
Val Asn Phe Leu Lys Asn Phe Trp Ser Asn Thr Thr Lys Glu Arg Asn
210 215 220
Ile Lys Asn Asn Leu Phe Ala Ala Glu Pro Pro Gln Val Tyr Ala Val
225 230 235 240
His Tyr Leu Gly Trp Lys Pro Trp Leu Cys Tyr Arg Asp Tyr Asp Cys
245 250 255
Asn Tyr Asp Val Asp Glu Gln Leu Val Tyr Ala Ser Asp Ala Ala His
260 265 270
Val Arg Trp Trp Lys Val His Asp Ser Met Asp Asp Ala Leu Gln Lys
275 280 285
Phe Cys Arg Leu Thr Lys Lys Arg Arg Thr Glu Ile Asn Trp Glu Arg
290 295 300
Arg Lys Ala Arg Leu Arg Gly Ser Thr Asp Tyr His Trp Lys Ile Asn
305 310 315 320
Val Thr Asp Pro Arg Arg Arg Arg Ser Tyr Leu Ile Gly
325 330

<210> 14
<211> 834
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)..(834)

<400> 14

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atg gct cct tcc aaa tct gca ctg ata cgc ttt aat cta gtc ttg ttg   48
Met Ala Pro Ser Lys Ser Ala Leu Ile Arg Phe Asn Leu Val Leu Leu
  1               5                  10                  15

gca gcg gag ctt cct ttg ttg gat gct ctt ttc gtg att gca ctc cca   96
Ala Ala Glu Leu Pro Leu Leu Asp Ala Leu Phe Val Ile Ala Leu Pro
             20                  25                  30

aga cta ata gat atc ttt ata ctg cta tgt gat cag gtg gtg aga gga   144
Arg Leu Ile Asp Ile Phe Ile Leu Leu Cys Asp Gln Val Val Arg Gly
         35                  40                  45

gtg aag atg caa gaa ctc gtt gaa gag aac gaa ata aac aag aaa gat   192
Val Lys Met Gln Glu Leu Val Glu Glu Asn Glu Ile Asn Lys Lys Asp
     50                  55                  60

ttg cta acc gct agt aac cag aca aag ctg gag gcg cca agc ttc atg   240
Leu Leu Thr Ala Ser Asn Gln Thr Lys Leu Glu Ala Pro Ser Phe Met
 65                  70                  75                  80

gaa gag att tta aca aga ggg tta gga aaa aca aag ata ggg atg gtg   288
Glu Glu Ile Leu Thr Arg Gly Leu Gly Lys Thr Lys Ile Gly Met Val
                 85                  90                  95

aac atg gaa gaa tgt gat ctt act aat tgg aaa cgt tat ggc gaa acg   336
Asn Met Glu Glu Cys Asp Leu Thr Asn Trp Lys Arg Tyr Gly Glu Thr
            100                 105                 110

gtt cac ata cat ttt gag cgt gtc tcg aag ctc ttc aaa tgg caa gac   384
Val His Ile His Phe Glu Arg Val Ser Lys Leu Phe Lys Trp Gln Asp
        115                 120                 125

ttg ttc ccc gag tgg ata gat gaa gag gaa gaa acc gag gtt ccc aca   432
Leu Phe Pro Glu Trp Ile Asp Glu Glu Glu Glu Thr Glu Val Pro Thr
    130                 135                 140

tgt cct gag ata cct atg ccc gat ttc gaa agc tta gag aag ttg gat   480
Cys Pro Glu Ile Pro Met Pro Asp Phe Glu Ser Leu Glu Lys Leu Asp
145                 150                 155                 160
```

```
ttg gta gta gtg aag ttg cct tgt aat tac cct gaa gaa ggg tgg aga   528
Leu Val Val Val Lys Leu Pro Cys Asn Tyr Pro Glu Glu Gly Trp Arg
                165                 170                 175

aga gag gtt ttg agg ttg caa gtg aac cta gtt gcg gct aac ttg gca   576
Arg Glu Val Leu Arg Leu Gln Val Asn Leu Val Ala Ala Asn Leu Ala
            180                 185                 190

gcc aag aaa ggg aag acg gat tgg aga tgg aaa agc aaa gtg ttg ttt   624
Ala Lys Lys Gly Lys Thr Asp Trp Arg Trp Lys Ser Lys Val Leu Phe
        195                 200                 205

tgg agc aaa tgt caa ccg atg att gag att ttc cgg tgt gat gat ttg   672
Trp Ser Lys Cys Gln Pro Met Ile Glu Ile Phe Arg Cys Asp Asp Leu
    210                 215                 220

gag aag aga gag gca gat tgg tgg ctg tat cgc cct gag gtg gtt agg   720
Glu Lys Arg Glu Ala Asp Trp Trp Leu Tyr Arg Pro Glu Val Val Arg
225                 230                 235                 240

tta caa cag aga ctc agt ttg cca gtc gga tct tgc aat ctt gct ctt   768
Leu Gln Gln Arg Leu Ser Leu Pro Val Gly Ser Cys Asn Leu Ala Leu
                245                 250                 255

cct ttg tgg gca cca caa ggt aaa att act ttc atg caa att aat ctt   816
Pro Leu Trp Ala Pro Gln Gly Lys Ile Thr Phe Met Gln Ile Asn Leu
            260                 265                 270

ctt gct aaa tat ttt tag                                         834
Leu Ala Lys Tyr Phe
        275
```

<210> 15
<211> 277
<212> PRT
<213> Arabidopsis thaliana

<400> 15

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Met Ala Pro Ser Lys Ser Ala Leu Ile Arg Phe Asn Leu Val Leu Leu
  1               5                  10                  15
Ala Ala Glu Leu Pro Leu Leu Asp Ala Leu Phe Val Ile Ala Leu Pro
             20                  25                  30
Arg Leu Ile Asp Ile Phe Ile Leu Leu Cys Asp Gln Val Val Arg Gly
         35                  40                  45
Val Lys Met Gln Glu Leu Val Glu Glu Asn Glu Ile Asn Lys Lys Asp
     50                  55                  60
Leu Leu Thr Ala Ser Asn Gln Thr Lys Leu Glu Ala Pro Ser Phe Met
 65                  70                  75                  80
Glu Glu Ile Leu Thr Arg Gly Leu Gly Lys Thr Lys Ile Gly Met Val
                 85                  90                  95
```

```
Asn Met Glu Glu Cys Asp Leu Thr Asn Trp Lys Arg Tyr Gly Glu Thr
            100                 105                 110
Val His Ile His Phe Glu Arg Val Ser Lys Leu Phe Lys Trp Gln Asp
        115                 120                 125
Leu Phe Pro Glu Trp Ile Asp Glu Glu Glu Glu Thr Glu Val Pro Thr
    130                 135                 140
Cys Pro Glu Ile Pro Met Pro Asp Phe Glu Ser Leu Glu Lys Leu Asp
145                 150                 155                 160
Leu Val Val Val Lys Leu Pro Cys Asn Tyr Pro Glu Glu Gly Trp Arg
                165                 170                 175
Arg Glu Val Leu Arg Leu Gln Val Asn Leu Val Ala Ala Asn Leu Ala
            180                 185                 190
Ala Lys Lys Gly Lys Thr Asp Trp Arg Trp Lys Ser Lys Val Leu Phe
        195                 200                 205
Trp Ser Lys Cys Gln Pro Met Ile Glu Ile Phe Arg Cys Asp Asp Leu
    210                 215                 220
Glu Lys Arg Glu Ala Asp Trp Trp Leu Tyr Arg Pro Glu Val Val Arg
225                 230                 235                 240
Leu Gln Gln Arg Leu Ser Leu Pro Val Gly Ser Cys Asn Leu Ala Leu
                245                 250                 255
Pro Leu Trp Ala Pro Gln Gly Lys Ile Thr Phe Met Gln Ile Asn Leu
            260                 265                 270
Leu Ala Lys Tyr Phe
        275
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<210> 16
<211> 383
<212> DNA
<213> Hordeum vulgare

<220>
<221> CDS
<222> (46)..(381)

<400> 16

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ttgaatctgc gggttggaag gtcagaataa ttgagaggat cggaa ccc gaa gcc gag 57
                                                  Pro Glu Ala Glu
                                                    1

cgt gat gct tac aat gag tgg aac tac agc aag ttc cgg ttg tgg cag   105
Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln
  5                  10                  15                  20

ctc acg gac tat gac aag atc ata ttc ata gat gct gat ctg ctc atc   153
Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala Asp Leu Leu Ile
                25                  30                  35

ttg agg aac att gat ttc ctg ttt aca atg cca gaa atc agt gca acc   201
Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu Ile Ser Ala Thr
            40                  45                  50
```

```
ggc aac aat gca aca ctc ttc aac tct ggt gtc atg gtc atc gaa ccc   249
Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met Val Ile Glu Pro
         55                  60                  65

tca aac tgc aca ttc cag ctg tta atg gag cac atc aat gag ata aca   297
Ser Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile Asn Glu Ile Thr
     70                  75                  80

tct tac aat ggt ggt gat cag ggc tac ttg aat gag ata ttc aca tgg   345
Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp
 85                  90                  95                  100

tgg cat cgg att ccc aag cac atg aac ttc ctg aag ca               383
Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys
                105                 110
```

<210> 17
<211> 112
<212> PRT
<213> Hordeum vulgare

<400> 17

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Pro Glu Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe
  1               5                  10                  15

Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala
             20                  25                  30

Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu
         35                  40                  45

Ile Ser Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met
     50                  55                  60

Val Ile Glu Pro Ser Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile
 65                  70                  75                  80

Asn Glu Ile Thr Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu
                 85                  90                  95

Ile Phe Thr Trp Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys
            100                 105                 110
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<210> 18
<211> 245
<212> DNA
<213> Hordeum vulgare

<220>
<221> CDS
<222> (52)..(243)

<400> 18
cgagcttgaa tctgcgggtt ggcaagtcag aataattgag aggatccgga a ccc gaa 57
Pro Glu
1

gcc gag cgt gat gct tac aat gag tgg aac tac agc aag ttc cgg ttg 105
Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu
5 10 15

tgg cag ctc acg gac tat gac aag atc ata ttc ata gat gct gat ctg 153
Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala Asp Leu
20 25 30

ctc atc ttg agg aac att gat ttc ctg ttt aca atg cca gaa atc agt 201
Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu Ile Ser
35 40 45 50

gca aac ggc aac aat gca aca ctc ttc aac tct ggt gtc atg gt 245
Ala Asn Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met
55 60

<210> 19
<211> 64
<212> PRT
<213> Hordeum vulgare

<400> 19
Pro Glu Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe
1 5 10 15

Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Ile Ile Phe Ile Asp Ala
20 25 30

Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Thr Met Pro Glu
35 40 45

Ile Ser Ala Asn Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met
50 55 60

<210> 20
<211> 1284
<212> DNA
<213> Triticum aestivum

<220>

<221> CDS
<222> (1)..(1284)

<400> 20

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acg cgt ccg ctc gcc ttc ttc ttc ctc gtt cta cat ggc cct cct gct   48
Thr Arg Pro Leu Ala Phe Phe Phe Leu Val Leu His Gly Pro Pro Ala
  1               5                  10                  15

cca ccc caa gta ctc cca cat cct cga ccg cgg cgc ctc ctc tct ggt   96
Pro Pro Gln Val Leu Pro His Pro Arg Pro Arg Arg Leu Leu Ser Gly
             20                  25                  30

ccg ctg cac ctt ccg cga cgc ctg ccc gtc cac gtc cca cct ctc acg   144
Pro Leu His Leu Pro Arg Arg Leu Pro Val His Val Pro Pro Leu Thr
         35                  40                  45

gaa ggt aag ccg gga gga aga tca gtg gcg gcg gcg aac aag gtg gtg   192
Glu Gly Lys Pro Gly Gly Arg Ser Val Ala Ala Ala Asn Lys Val Val
     50                  55                  60

gcg acg gag cgg atc gtg aac gcg ggg cgc gcg ccg acc atg ttc aac   240
Ala Thr Glu Arg Ile Val Asn Ala Gly Arg Ala Pro Thr Met Phe Asn
 65                  70                  75                  80

gag ctg cgc ggc cgg ctg cgg atg ggc ctg gtg aac atc ggc cgc gac   288
Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val Asn Ile Gly Arg Asp
                 85                  90                  95

gag ctg ctg gcg ctg ggc gtg gag gga gac gcc gtg ggc gtg gac ttc   336
Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala Val Gly Val Asp Phe
            100                 105                 110

gac cgc gtg tcg gac gtg ttc cgg tgg tca gac ctg ttc ccg gag tgg   384
Asp Arg Val Ser Asp Val Phe Arg Trp Ser Asp Leu Phe Pro Glu Trp
        115                 120                 125

atc gac gag gag gag gag gac ggc gtc ccc tcc tgc ccg gag atc ccc   432
Ile Asp Glu Glu Glu Glu Asp Gly Val Pro Ser Cys Pro Glu Ile Pro
    130                 135                 140

atg ccg gac ttc tcc cgg tac gac gac gac ggc gtg gac gtg gtg gtg   480
Met Pro Asp Phe Ser Arg Tyr Asp Asp Asp Gly Val Asp Val Val Val
145                 150                 155                 160

gcg gcg ctg ccg tgc aac cgg acg gcg gtc cgg ggg tgg aac cgc gac   528
Ala Ala Leu Pro Cys Asn Arg Thr Ala Val Arg Gly Trp Asn Arg Asp
                165                 170                 175

gtg ttc agg ctg cag gtg cac ctg gtg gcg gcg cac atg gcg gcg cgg   576
Val Phe Arg Leu Gln Val His Leu Val Ala Ala His Met Ala Ala Arg
            180                 185                 190
```

```
aag tgg gcg gcg cga cgg cgc cgg ccg ggt gcg cgt ggt gct gcg gag   624
Lys Trp Ala Ala Arg Arg Arg Arg Pro Gly Ala Arg Gly Ala Ala Glu
        195                 200                 205

cga gtg cga gcc gat gat gga cct gtt ccg gtg cga cga gtc cgt ggg   672
Arg Val Arg Ala Asp Asp Gly Pro Val Pro Val Arg Arg Val Arg Gly
    210                 215                 220

gcg gga ggg gga ctg gtg gat gta cag cgt cga cgc gcc gcg cat gga   720
Ala Gly Gly Gly Leu Val Asp Val Gln Arg Arg Arg Ala Ala His Gly
225                 230                 235                 240

gga gaa gct ccg gct gcc cat cgg ctc ctg caa cct cgc cgc tgc cgc   768
Gly Glu Ala Pro Ala Ala His Arg Leu Leu Gln Pro Arg Arg Cys Arg
                245                 250                 255

tct ggg ggc caa cag gca tcc acg agg tgt tca acg cgt cag acc taa   816
Ser Gly Gly Gln Gln Ala Ser Thr Arg Cys Ser Thr Arg Gln Thr
            260                 265                 270

cag cgg tgg acg ccg gca gcc agc ggc gcg agg cgt acg cga ctg gtg   864
Gln Arg Trp Thr Pro Ala Ala Ser Gly Ala Arg Arg Thr Arg Leu Val
        275                 280                 285

ctg cac tcg tcc gac cga tac ctg tgc ggc gcc atc gtg ctg gcg cag   912
Leu His Ser Ser Asp Arg Tyr Leu Cys Gly Ala Ile Val Leu Ala Gln
    290                 295                 300

agc atc cgg cgg tcg ggc tcc acc cgc gac atg gtc ctc ctc cac gac   960
Ser Ile Arg Arg Ser Gly Ser Thr Arg Asp Met Val Leu Leu His Asp
305                 310                 315                 320

cac acc gtc tcc aag ccg gcc ctc cgc gcg ctg gtc gcc gcc ggc tgg  1008
His Thr Val Ser Lys Pro Ala Leu Arg Ala Leu Val Ala Ala Gly Trp
                325                 330                 335

atc ccg cgc agg atc cgg cgc atc cgc aac ccg cgc gcg gag cgg ggc  1056
Ile Pro Arg Arg Ile Arg Arg Ile Arg Asn Pro Arg Ala Glu Arg Gly
            340                 345                 350

tcc tac aac gag tac aac tac agc aag ttc cgg ctg tgg cag ctg acg  1104
Ser Tyr Asn Glu Tyr Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr
        355                 360                 365

gag tac ttc cgc gtc gtc ttc atc gac gcc gac atc ctc gtc ctc cgc  1152
Glu Tyr Phe Arg Val Val Phe Ile Asp Ala Asp Ile Leu Val Leu Arg
    370                 375                 380

tcc ctc gac gcg ctc ttc cgc ttc ccg cag atc tcc gcc ggg ggc aac  1200
Ser Leu Asp Ala Leu Phe Arg Phe Pro Gln Ile Ser Ala Gly Gly Asn
385                 390                 395                 400
```

```
gac ggc tcc ctc ttc aac tcg ggg aac atg gtg ctc gag ccg tcg gcg   1248
Asp Gly Ser Leu Phe Asn Ser Gly Asn Met Val Leu Glu Pro Ser Ala
                405                 410                 415

tgc acc ttc gag gcg ctc gtc cgg ggg cgg cgc aca                   1284
Cys Thr Phe Glu Ala Leu Val Arg Gly Arg Arg Thr
            420                 425
```

<210> 21
<211> 271
<212> PRT
<213> Triticum aestivum

<400> 21

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Thr Arg Pro Leu Ala Phe Phe Phe Leu Val Leu His Gly Pro Pro Ala
  1               5                  10                  15
Pro Pro Gln Val Leu Pro His Pro Arg Pro Arg Arg Leu Leu Ser Gly
             20                  25                  30
Pro Leu His Leu Pro Arg Arg Leu Pro Val His Val Pro Pro Leu Thr
         35                  40                  45
Glu Gly Lys Pro Gly Gly Arg Ser Val Ala Ala Ala Asn Lys Val Val
     50                  55                  60
Ala Thr Glu Arg Ile Val Asn Ala Gly Arg Ala Pro Thr Met Phe Asn
 65                  70                  75                  80
Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val Asn Ile Gly Arg Asp
                 85                  90                  95
Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala Val Gly Val Asp Phe
            100                 105                 110
Asp Arg Val Ser Asp Val Phe Arg Trp Ser Asp Leu Phe Pro Glu Trp
        115                 120                 125
Ile Asp Glu Glu Glu Glu Asp Gly Val Pro Ser Cys Pro Glu Ile Pro
    130                 135                 140
Met Pro Asp Phe Ser Arg Tyr Asp Asp Asp Gly Val Asp Val Val Val
145                 150                 155                 160
Ala Ala Leu Pro Cys Asn Arg Thr Ala Val Arg Gly Trp Asn Arg Asp
                165                 170                 175
Val Phe Arg Leu Gln Val His Leu Val Ala Ala His Met Ala Ala Arg
            180                 185                 190
Lys Trp Ala Ala Arg Arg Arg Arg Pro Gly Ala Arg Gly Ala Ala Glu
        195                 200                 205
Arg Val Arg Ala Asp Asp Gly Pro Val Pro Val Arg Arg Val Arg Gly
    210                 215                 220
Ala Gly Gly Gly Leu Val Asp Val Gln Arg Arg Arg Ala Ala His Gly
225                 230                 235                 240
Gly Glu Ala Pro Ala Ala His Arg Leu Leu Gln Pro Arg Arg Cys Arg
                245                 250                 255
Ser Gly Gly Gln Gln Ala Ser Thr Arg Cys Ser Thr Arg Gln Thr
            260                 265                 270
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<210> 22

<211> 156
<212> PRT
<213> Triticum aestivum

<400> 22
Gln Arg Trp Thr Pro Ala Ala Ser Gly Ala Arg Arg Thr Arg Leu Val
1 5 10 15
Leu His Ser Ser Asp Arg Tyr Leu Cys Gly Ala Ile Val Leu Ala Gln
20 25 30
Ser Ile Arg Arg Ser Gly Ser Thr Arg Asp Met Val Leu Leu His Asp
35 40 45
His Thr Val Ser Lys Pro Ala Leu Arg Ala Leu Val Ala Ala Gly Trp
50 55 60
Ile Pro Arg Arg Ile Arg Arg Ile Arg Asn Pro Arg Ala Glu Arg Gly
65 70 75 80
Ser Tyr Asn Glu Tyr Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr
85 90 95
Glu Tyr Phe Arg Val Val Phe Ile Asp Ala Asp Ile Leu Val Leu Arg
100 105 110
Ser Leu Asp Ala Leu Phe Arg Phe Pro Gln Ile Ser Ala Gly Gly Asn
115 120 125
Asp Gly Ser Leu Phe Asn Ser Gly Asn Met Val Leu Glu Pro Ser Ala
130 135 140
Cys Thr Phe Glu Ala Leu Val Arg Gly Arg Arg Thr
145 150 155

<210> 23
<211> 2028
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)..(1854)

<400> 23
atg ata cct tcc tca agt ccc atg gag tca aga cat cga ctc tcg ttc 48
Met Ile Pro Ser Ser Ser Pro Met Glu Ser Arg His Arg Leu Ser Phe
1 5 10 15

tca aat gag aag aca agt agg agg aga ttt caa aga att gag aag ggt 96
Ser Asn Glu Lys Thr Ser Arg Arg Arg Phe Gln Arg Ile Glu Lys Gly
20 25 30

gtc aag ttc aac act ctg aaa ctt gtg ttg att tgt ata atg ctt gga 144
Val Lys Phe Asn Thr Leu Lys Leu Val Leu Ile Cys Ile Met Leu Gly
35 40 45

gct ttg ttc acg atc tac cgt ttt cgt tat cca ccg cta caa att cct 192
Ala Leu Phe Thr Ile Tyr Arg Phe Arg Tyr Pro Pro Leu Gln Ile Pro

```
     50                  55                  60
gaa att cca act agt ttt ggt ctt act act gat cct cgc tat gta gct   240
Glu Ile Pro Thr Ser Phe Gly Leu Thr Thr Asp Pro Arg Tyr Val Ala
 65                  70                  75                  80

aca gct gag atc aac tgg aac cat atg tca aat ctt gtt gag aag cac   288
Thr Ala Glu Ile Asn Trp Asn His Met Ser Asn Leu Val Glu Lys His
                85                  90                  95

gta ttt ggt aga agc gag tat caa gga att ggt ctt ata aat ctt aac   336
Val Phe Gly Arg Ser Glu Tyr Gln Gly Ile Gly Leu Ile Asn Leu Asn
            100                 105                 110

gat aac gag att gat cga ttc aag gag gta acg aaa tct gac tgt gat   384
Asp Asn Glu Ile Asp Arg Phe Lys Glu Val Thr Lys Ser Asp Cys Asp
        115                 120                 125

cat gta gct ttg cat cta gat tat gct gca aag aac ata aca tgg gaa   432
His Val Ala Leu His Leu Asp Tyr Ala Ala Lys Asn Ile Thr Trp Glu
    130                 135                 140

tct tta tac ccg gaa tgg att gat gaa gtt gaa gaa ttc gaa gtc cct   480
Ser Leu Tyr Pro Glu Trp Ile Asp Glu Val Glu Glu Phe Glu Val Pro
145                 150                 155                 160

act tgt cct tct ctg cct ttg att caa att cct ggc aag cct cgg att   528
Thr Cys Pro Ser Leu Pro Leu Ile Gln Ile Pro Gly Lys Pro Arg Ile
                165                 170                 175

gat ctt gta att gcc aag ctt ccg tgt gat aaa tca gga aaa tgg tct   576
Asp Leu Val Ile Ala Lys Leu Pro Cys Asp Lys Ser Gly Lys Trp Ser
            180                 185                 190

aga gat gtg gct cgc ttg cat tta caa ctt gca gca gct cga gtg gcg   624
Arg Asp Val Ala Arg Leu His Leu Gln Leu Ala Ala Ala Arg Val Ala
        195                 200                 205

gct tct tct aaa gga ctt cat aat gtt cat gtg att ttg gta tct gat   672
Ala Ser Ser Lys Gly Leu His Asn Val His Val Ile Leu Val Ser Asp
    210                 215                 220

tgc ttt cca ata ccg aat ctt ttt acg ggt caa gaa ctt gtt gcc cgt   720
Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly Gln Glu Leu Val Ala Arg
225                 230                 235                 240

caa gga aac ata tgg ctg tat aag cct aat ctt cac cag cta aga caa   768
Gln Gly Asn Ile Trp Leu Tyr Lys Pro Asn Leu His Gln Leu Arg Gln
                245                 250                 255

aag tta cag ctt cct gtt ggt tcc tgt gaa ctt tct gtt cct ctt caa   816
Lys Leu Gln Leu Pro Val Gly Ser Cys Glu Leu Ser Val Pro Leu Gln
            260                 265                 270
```

```
gct aaa gat aat ttc tac tcc gca ggt gca aag aaa gaa gct tac gcg   864
Ala Lys Asp Asn Phe Tyr Ser Ala Gly Ala Lys Lys Glu Ala Tyr Ala
        275                 280                 285

act atc ttg cat tct gcc caa ttt tat gtc tgt gga gcc att gca gct   912
Thr Ile Leu His Ser Ala Gln Phe Tyr Val Cys Gly Ala Ile Ala Ala
    290                 295                 300

gca cag agc att cga atg tca ggc tct act cgt gat ctg gtc ata ctt   960
Ala Gln Ser Ile Arg Met Ser Gly Ser Thr Arg Asp Leu Val Ile Leu
305                 310                 315                 320

gtt gat gaa acg ata agc gaa tac cat aaa agt ggc ttg gta gct gct   1008
Val Asp Glu Thr Ile Ser Glu Tyr His Lys Ser Gly Leu Val Ala Ala
                325                 330                 335

gga tgg aag att caa atg ttt caa aga atc agg aac ccg aat gct gta   1056
Gly Trp Lys Ile Gln Met Phe Gln Arg Ile Arg Asn Pro Asn Ala Val
            340                 345                 350

cca aat gcc tac aac gaa tgg aac tac agc aag ttt cgt ctt tgg caa   1104
Pro Asn Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln
        355                 360                 365

ctg act gaa tac agt aag atc atc ttc atc gat gca gac atg ctt atc   1152
Leu Thr Glu Tyr Ser Lys Ile Ile Phe Ile Asp Ala Asp Met Leu Ile
    370                 375                 380

ctg aga aac att gat ttc ctc ttc gag ttc cct gag ata tca gca act   1200
Leu Arg Asn Ile Asp Phe Leu Phe Glu Phe Pro Glu Ile Ser Ala Thr
385                 390                 395                 400

gga aac aat gct acg ctc ttc aac tct ggt cta atg gtg gtt gag cca   1248
Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Leu Met Val Val Glu Pro
                405                 410                 415

tct aat tca aca ttc cag tta cta atg gat aac att aat gaa gtt gtg   1296
Ser Asn Ser Thr Phe Gln Leu Leu Met Asp Asn Ile Asn Glu Val Val
            420                 425                 430

tct tac aac gga gga gac caa ggt tac ctt aac gag ata ttc aca tgg   1344
Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp
        435                 440                 445

tgg cat cgg att cca aaa cac atg aat ttc ttg aag cat ttc tgg gaa   1392
Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu
    450                 455                 460

gga gac gaa cct gag att aaa aaa atg aag acg agt cta ttt gga gct   1440
Gly Asp Glu Pro Glu Ile Lys Lys Met Lys Thr Ser Leu Phe Gly Ala
465                 470                 475                 480
```

```
gat cct ccg atc cta tac gtt ctt cat tac cta ggt tat aac aaa ccc   1488
Asp Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Tyr Asn Lys Pro
                485                 490                 495

tgg tta tgc ttc aga gac tat gac tgc aat tgg aat gtc gat att ttc   1536
Trp Leu Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Asp Ile Phe
            500                 505                 510

cag gaa ttt gct agt gac gag gct cat aaa acc tgg tgg aga gtg cac   1584
Gln Glu Phe Ala Ser Asp Glu Ala His Lys Thr Trp Trp Arg Val His
        515                 520                 525

gac gca atg cct gaa aac ttg cat aag ttc tgt cta cta aga tcg aaa   1632
Asp Ala Met Pro Glu Asn Leu His Lys Phe Cys Leu Leu Arg Ser Lys
    530                 535                 540

cag aag gcg caa ctt gaa tgg gat agg aga caa gca gag aaa ggg aac   1680
Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg Gln Ala Glu Lys Gly Asn
545                 550                 555                 560

tac aaa gat gga cat tgg aag ata aag atc aaa gac aag aga ctt aag   1728
Tyr Lys Asp Gly His Trp Lys Ile Lys Ile Lys Asp Lys Arg Leu Lys
                565                 570                 575

act tgt ttc gaa gat ttc tgc ttt tgg gag agt atg ctt tgg cat tgg   1776
Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu Ser Met Leu Trp His Trp
            580                 585                 590

ggt gag acg aac tct acc aac aat tct tcc acc acc acc act tca tca   1824
Gly Glu Thr Asn Ser Thr Asn Asn Ser Ser Thr Thr Thr Thr Ser Ser
        595                 600                 605

ccg ccg cat aaa acc gct ctc cct tcc ctg tgaattcttt tggctttctg     1874
Pro Pro His Lys Thr Ala Leu Pro Ser Leu
    610                 615

gtttggtaca aattactctg cctttcgcca accaaatgtg ggttggatat gttcttttgt 1934

ttttttatta tcagcttgaa acctgtatac gaatcccaga aacaatgtaa tcatgagggg 1994

ataaaggaat gaaagacaaa taaagaattt acag                             2028
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<210> 24
<211> 618
<212> PRT
<213> Arabidopsis thaliana

<400> 24

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Met Ile Pro Ser Ser Ser Pro Met Glu Ser Arg His Arg Leu Ser Phe
  1               5                  10                  15
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Ser Asn Glu Lys Thr Ser Arg Arg Arg Phe Gln Arg Ile Glu Lys Gly
            20                  25                  30

Val Lys Phe Asn Thr Leu Lys Leu Val Leu Ile Cys Ile Met Leu Gly
        35                  40                  45

Ala Leu Phe Thr Ile Tyr Arg Phe Arg Tyr Pro Pro Leu Gln Ile Pro
    50                  55                  60

Glu Ile Pro Thr Ser Phe Gly Leu Thr Thr Asp Pro Arg Tyr Val Ala
65                  70                  75                  80

Thr Ala Glu Ile Asn Trp Asn His Met Ser Asn Leu Val Glu Lys His
                85                  90                  95

Val Phe Gly Arg Ser Glu Tyr Gln Gly Ile Gly Leu Ile Asn Leu Asn
            100                 105                 110

Asp Asn Glu Ile Asp Arg Phe Lys Glu Val Thr Lys Ser Asp Cys Asp
        115                 120                 125

His Val Ala Leu His Leu Asp Tyr Ala Ala Lys Asn Ile Thr Trp Glu
    130                 135                 140

Ser Leu Tyr Pro Glu Trp Ile Asp Glu Val Glu Glu Phe Glu Val Pro
145                 150                 155                 160

Thr Cys Pro Ser Leu Pro Leu Ile Gln Ile Pro Gly Lys Pro Arg Ile
                165                 170                 175

Asp Leu Val Ile Ala Lys Leu Pro Cys Asp Lys Ser Gly Lys Trp Ser
            180                 185                 190

Arg Asp Val Ala Arg Leu His Leu Gln Leu Ala Ala Ala Arg Val Ala
        195                 200                 205

Ala Ser Ser Lys Gly Leu His Asn Val His Val Ile Leu Val Ser Asp
    210                 215                 220

Cys Phe Pro Ile Pro Asn Leu Phe Thr Gly Gln Glu Leu Val Ala Arg
225                 230                 235                 240

Gln Gly Asn Ile Trp Leu Tyr Lys Pro Asn Leu His Gln Leu Arg Gln
                245                 250                 255

Lys Leu Gln Leu Pro Val Gly Ser Cys Glu Leu Ser Val Pro Leu Gln
            260                 265                 270

Ala Lys Asp Asn Phe Tyr Ser Ala Gly Ala Lys Lys Glu Ala Tyr Ala
        275                 280                 285
```

```
Thr Ile Leu His Ser Ala Gln Phe Tyr Val Cys Gly Ala Ile Ala Ala
    290                 295                 300

Ala Gln Ser Ile Arg Met Ser Gly Ser Thr Arg Asp Leu Val Ile Leu
305                 310                 315                 320

Val Asp Glu Thr Ile Ser Glu Tyr His Lys Ser Gly Leu Val Ala Ala
                325                 330                 335

Gly Trp Lys Ile Gln Met Phe Gln Arg Ile Arg Asn Pro Asn Ala Val
            340                 345                 350

Pro Asn Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln
        355                 360                 365

Leu Thr Glu Tyr Ser Lys Ile Ile Phe Ile Asp Ala Asp Met Leu Ile
    370                 375                 380

Leu Arg Asn Ile Asp Phe Leu Phe Glu Phe Pro Glu Ile Ser Ala Thr
385                 390                 395                 400

Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly Leu Met Val Val Glu Pro
                405                 410                 415

Ser Asn Ser Thr Phe Gln Leu Leu Met Asp Asn Ile Asn Glu Val Val
            420                 425                 430

Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp
        435                 440                 445

Trp His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu
    450                 455                 460

Gly Asp Glu Pro Glu Ile Lys Lys Met Lys Thr Ser Leu Phe Gly Ala
465                 470                 475                 480

Asp Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Tyr Asn Lys Pro
                485                 490                 495

Trp Leu Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Asp Ile Phe
            500                 505                 510

Gln Glu Phe Ala Ser Asp Glu Ala His Lys Thr Trp Trp Arg Val His
        515                 520                 525

Asp Ala Met Pro Glu Asn Leu His Lys Phe Cys Leu Leu Arg Ser Lys
    530                 535                 540

Gln Lys Ala Gln Leu Glu Trp Asp Arg Arg Gln Ala Glu Lys Gly Asn
545                 550                 555                 560

Tyr Lys Asp Gly His Trp Lys Ile Lys Ile Lys Asp Lys Arg Leu Lys
```

565 570 575

Thr Cys Phe Glu Asp Phe Cys Phe Trp Glu Ser Met Leu Trp His Trp
580 585 590

Gly Glu Thr Asn Ser Thr Asn Asn Ser Ser Thr Thr Thr Thr Ser Ser
595 600 605

Pro Pro His Lys Thr Ala Leu Pro Ser Leu
610 615

<210> 25
<211> 1845
<212> DNA
<213> Oryza sativa

<220>
<221> CDS
<222> (1)..(1845)

<400> 25

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atg ggg gtg acg ggc ggc gcc ggg gag gcc gtc aag ccg tcg tcg tcg   48
Met Gly Val Thr Gly Gly Ala Gly Glu Ala Val Lys Pro Ser Ser Ser
  1               5                  10                  15

tcg tcg ttg tcg ccg gtg gcg ggg ctg agg gcg gcg gcc atc gtg aag   96
Ser Ser Leu Ser Pro Val Ala Gly Leu Arg Ala Ala Ala Ile Val Lys
             20                  25                  30

ctg aac gcg gcg ttc ctc gcc ttc ttc ttc ctc gcg tac atg gcg ctc   144
Leu Asn Ala Ala Phe Leu Ala Phe Phe Phe Leu Ala Tyr Met Ala Leu
         35                  40                  45

ctc ctc cac ccc aag tac tcc tac ctc ctc gac cgc ggc gcc gcc tcc   192
Leu Leu His Pro Lys Tyr Ser Tyr Leu Leu Asp Arg Gly Ala Ala Ser
     50                  55                  60

tcc ctc gtc cgc tgc acc gcc ttc cgc gac gcc tgc acc ccg gcg acg   240
Ser Leu Val Arg Cys Thr Ala Phe Arg Asp Ala Cys Thr Pro Ala Thr
 65                  70                  75                  80

acg acc acc gcc cag ctc tct cgg aag ctg gga ggc gtg gcg gcg aac   288
Thr Thr Thr Ala Gln Leu Ser Arg Lys Leu Gly Gly Val Ala Ala Asn
                 85                  90                  95

aag gcg gtg gcg gcg gcg gcg gag agg atc gtg aac gcc ggg agg gcg   336
Lys Ala Val Ala Ala Ala Ala Glu Arg Ile Val Asn Ala Gly Arg Ala
            100                 105                 110

ccg gcg atg ttc gac gag ctc cgt ggg cgg ctg cgg atg ggc ctg gtg   384
```

```
Pro Ala Met Phe Asp Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val
        115                 120                 125

aac atc ggc cgc gac gag ctg ctg gcg ctc ggc gtg gag ggc gac gcc   432
Asn Ile Gly Arg Asp Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala
    130                 135                 140

gtc ggc gtc gac ttc gag cgc gtc tcc gac atg ttc cgg tgg tcg gac   480
Val Gly Val Asp Phe Glu Arg Val Ser Asp Met Phe Arg Trp Ser Asp
145                 150                 155                 160

ctc ttc ccg gag tgg atc gac gag gag gag gac gac gag ggc ccg tcc   528
Leu Phe Pro Glu Trp Ile Asp Glu Glu Glu Asp Asp Glu Gly Pro Ser
                165                 170                 175

tgc ccg gag ctc ccc atg ccg gac ttc tcc cgg tac ggc gac gtc gac   576
Cys Pro Glu Leu Pro Met Pro Asp Phe Ser Arg Tyr Gly Asp Val Asp
            180                 185                 190

gtg gtg gtg gcg tcg ctg ccg tgc aac cgt tcg gac gcc gcg tgg aac   624
Val Val Val Ala Ser Leu Pro Cys Asn Arg Ser Asp Ala Ala Trp Asn
        195                 200                 205

cgc gac gtg ttc agg ctg cag gtg cac ctc gtg acg gcg cac atg gcg   672
Arg Asp Val Phe Arg Leu Gln Val His Leu Val Thr Ala His Met Ala
    210                 215                 220

gcg cgc aag ggg ctg cgg cac gac gcc ggc ggc ggc ggc ggc ggc ggg   720
Ala Arg Lys Gly Leu Arg His Asp Ala Gly Gly Gly Gly Gly Gly Gly
225                 230                 235                 240

cgg gtg cgc gtg gtg gtg cgc agc gag tgc gag ccc atg atg gac ttg   768
Arg Val Arg Val Val Val Arg Ser Glu Cys Glu Pro Met Met Asp Leu
                245                 250                 255

ttc cgg tgc gac gag gcg gtg ggg agg gac ggc gag tgg tgg atg tac   816
Phe Arg Cys Asp Glu Ala Val Gly Arg Asp Gly Glu Trp Trp Met Tyr
            260                 265                 270

atg gtc gac gtc gag cgg ctg gag gag aag ctc cgg ctt cct gtc ggc   864
Met Val Asp Val Glu Arg Leu Glu Glu Lys Leu Arg Leu Pro Val Gly
        275                 280                 285

tca tgc aac ctc gcc cta cct ctg tgg gga ccc gga ggt atc cag gaa   912
Ser Cys Asn Leu Ala Leu Pro Leu Trp Gly Pro Gly Gly Ile Gln Glu
    290                 295                 300

gtg ttc aac gtg tcg gag ctg acg gcg gcg gcg gca acg gcg ggg cgg   960
Val Phe Asn Val Ser Glu Leu Thr Ala Ala Ala Ala Thr Ala Gly Arg
305                 310                 315                 320

ccg cgg cgg gag gcg tac gcg acg gtg ctc cac tcg tcg gac acg tac  1008
```

```
Pro Arg Arg Glu Ala Tyr Ala Thr Val Leu His Ser Ser Asp Thr Tyr
                325                 330                 335

ctg tgc ggc gcg atc gtg ctg gcg cag agc atc cgg cgc gcc ggg tcg   1056
Leu Cys Gly Ala Ile Val Leu Ala Gln Ser Ile Arg Arg Ala Gly Ser
            340                 345                 350

acg cgc gac ctc gtc ctc ctc cac gac cac acc gtg tcg aag ccg gcg   1104
Thr Arg Asp Leu Val Leu Leu His Asp His Thr Val Ser Lys Pro Ala
        355                 360                 365

ctg gcg gcg ctg gtc gcc gcc ggc tgg acc ccg cgc aag atc aag cgc   1152
Leu Ala Ala Leu Val Ala Ala Gly Trp Thr Pro Arg Lys Ile Lys Arg
    370                 375                 380

atc cgc aac ccg cgc gcg gag cgc ggc acc tac aac gag tac aac tac   1200
Ile Arg Asn Pro Arg Ala Glu Arg Gly Thr Tyr Asn Glu Tyr Asn Tyr
385                 390                 395                 400

agc aag ttc cgg ctg tgg cag ctc acc gac tac gac cgc gtg gtg ttc   1248
Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Arg Val Val Phe
                405                 410                 415

gtc gac gcc gac atc ctc gtc ctc cgc gac ctc gac gcc ctc ttc ggc   1296
Val Asp Ala Asp Ile Leu Val Leu Arg Asp Leu Asp Ala Leu Phe Gly
            420                 425                 430

ttc ccg cag ctg acg gcg gtg ggc aac gac ggc tcg ctc ttc aac tcc   1344
Phe Pro Gln Leu Thr Ala Val Gly Asn Asp Gly Ser Leu Phe Asn Ser
        435                 440                 445

ggg gtg atg gtg atc gag ccg tcg cag tgc acg ttc cag tcg ctg atc   1392
Gly Val Met Val Ile Glu Pro Ser Gln Cys Thr Phe Gln Ser Leu Ile
    450                 455                 460

cgg cag cgg cgg acc atc cgg tcc tac aac ggc ggc gat cag ggg ttc   1440
Arg Gln Arg Arg Thr Ile Arg Ser Tyr Asn Gly Gly Asp Gln Gly Phe
465                 470                 475                 480

ctg aac gag gtg ttc gtc tgg tgg cac cgg ctg ccg cgg cgg gtg aac   1488
Leu Asn Glu Val Phe Val Trp Trp His Arg Leu Pro Arg Arg Val Asn
                485                 490                 495

tac ctc aag aac ttc tgg gcg aac act acg gcg gag cgg gcg ctc aag   1536
Tyr Leu Lys Asn Phe Trp Ala Asn Thr Thr Ala Glu Arg Ala Leu Lys
            500                 505                 510

gag cgg ctg ttc cgg gcg gat ccc gcg gag gtg tgg tcg atc cac tac   1584
Glu Arg Leu Phe Arg Ala Asp Pro Ala Glu Val Trp Ser Ile His Tyr
        515                 520                 525

ctg ggg ctg aag ccg tgg acg tgc tac cgc gac tac gac tgc aac tgg   1632
```

```
Leu Gly Leu Lys Pro Trp Thr Cys Tyr Arg Asp Tyr Asp Cys Asn Trp
    530                 535                 540

aac atc ggc gac cag cgg gtg tac gcc agc gac gcc gcg cac gcg cgg   1680
Asn Ile Gly Asp Gln Arg Val Tyr Ala Ser Asp Ala Ala His Ala Arg
545                 550                 555                 560

tgg tgg cag gtg tac gac gac atg ggg gag gcc atg cgc tcg ccg tgc   1728
Trp Trp Gln Val Tyr Asp Asp Met Gly Glu Ala Met Arg Ser Pro Cys
                565                 570                 575

cgc ctg tcg gag cgg agg aag atc gag atc gcc tgg gac cga cac ctc   1776
Arg Leu Ser Glu Arg Arg Lys Ile Glu Ile Ala Trp Asp Arg His Leu
            580                 585                 590

gcc gag gag gcc ggc ttc tcc gac cac cac tgg aag atc aac atc acc   1824
Ala Glu Glu Ala Gly Phe Ser Asp His His Trp Lys Ile Asn Ile Thr
        595                 600                 605

gac ccc cgc aag tgg gag tag                                      1845
Asp Pro Arg Lys Trp Glu  *
    610
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<210> 26
<211> 614
<212> PRT
<213> Oryza sativa

<400> 26

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Met Gly Val Thr Gly Gly Ala Gly Glu Ala Val Lys Pro Ser Ser Ser
  1               5                  10                  15
Ser Ser Leu Ser Pro Val Ala Gly Leu Arg Ala Ala Ala Ile Val Lys
             20                  25                  30
Leu Asn Ala Ala Phe Leu Ala Phe Phe Phe Leu Ala Tyr Met Ala Leu
         35                  40                  45
Leu Leu His Pro Lys Tyr Ser Tyr Leu Leu Asp Arg Gly Ala Ala Ser
     50                  55                  60
Ser Leu Val Arg Cys Thr Ala Phe Arg Asp Ala Cys Thr Pro Ala Thr
 65                  70                  75                  80
Thr Thr Thr Ala Gln Leu Ser Arg Lys Leu Gly Gly Val Ala Ala Asn
                 85                  90                  95
Lys Ala Val Ala Ala Ala Ala Glu Arg Ile Val Asn Ala Gly Arg Ala
            100                 105                 110
Pro Ala Met Phe Asp Glu Leu Arg Gly Arg Leu Arg Met Gly Leu Val
        115                 120                 125
Asn Ile Gly Arg Asp Glu Leu Leu Ala Leu Gly Val Glu Gly Asp Ala
    130                 135                 140
Val Gly Val Asp Phe Glu Arg Val Ser Asp Met Phe Arg Trp Ser Asp
145                 150                 155                 160
Leu Phe Pro Glu Trp Ile Asp Glu Glu Glu Asp Asp Glu Gly Pro Ser
                165                 170                 175
```

```
Cys Pro Glu Leu Pro Met Pro Asp Phe Ser Arg Tyr Gly Asp Val Asp
            180                 185                 190
Val Val Val Ala Ser Leu Pro Cys Asn Arg Ser Asp Ala Ala Trp Asn
        195                 200                 205
Arg Asp Val Phe Arg Leu Gln Val His Leu Val Thr Ala His Met Ala
    210                 215                 220
Ala Arg Lys Gly Leu Arg His Asp Ala Gly Gly Gly Gly Gly Gly Gly
225                 230                 235                 240
Arg Val Arg Val Val Val Arg Ser Glu Cys Glu Pro Met Met Asp Leu
                245                 250                 255
Phe Arg Cys Asp Glu Ala Val Gly Arg Asp Gly Glu Trp Trp Met Tyr
            260                 265                 270
Met Val Asp Val Glu Arg Leu Glu Glu Lys Leu Arg Leu Pro Val Gly
        275                 280                 285
Ser Cys Asn Leu Ala Leu Pro Leu Trp Gly Pro Gly Gly Ile Gln Glu
    290                 295                 300
Val Phe Asn Val Ser Glu Leu Thr Ala Ala Ala Ala Thr Ala Gly Arg
305                 310                 315                 320
Pro Arg Arg Glu Ala Tyr Ala Thr Val Leu His Ser Ser Asp Thr Tyr
                325                 330                 335
Leu Cys Gly Ala Ile Val Leu Ala Gln Ser Ile Arg Arg Ala Gly Ser
            340                 345                 350
Thr Arg Asp Leu Val Leu Leu His Asp His Thr Val Ser Lys Pro Ala
        355                 360                 365
Leu Ala Ala Leu Val Ala Ala Gly Trp Thr Pro Arg Lys Ile Lys Arg
    370                 375                 380
Ile Arg Asn Pro Arg Ala Glu Arg Gly Thr Tyr Asn Glu Tyr Asn Tyr
385                 390                 395                 400
Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Arg Val Val Phe
                405                 410                 415
Val Asp Ala Asp Ile Leu Val Leu Arg Asp Leu Asp Ala Leu Phe Gly
            420                 425                 430
Phe Pro Gln Leu Thr Ala Val Gly Asn Asp Gly Ser Leu Phe Asn Ser
        435                 440                 445
Gly Val Met Val Ile Glu Pro Ser Gln Cys Thr Phe Gln Ser Leu Ile
    450                 455                 460
Arg Gln Arg Arg Thr Ile Arg Ser Tyr Asn Gly Gly Asp Gln Gly Phe
465                 470                 475                 480
Leu Asn Glu Val Phe Val Trp Trp His Arg Leu Pro Arg Arg Val Asn
                485                 490                 495
Tyr Leu Lys Asn Phe Trp Ala Asn Thr Thr Ala Glu Arg Ala Leu Lys
            500                 505                 510
Glu Arg Leu Phe Arg Ala Asp Pro Ala Glu Val Trp Ser Ile His Tyr
        515                 520                 525
Leu Gly Leu Lys Pro Trp Thr Cys Tyr Arg Asp Tyr Asp Cys Asn Trp
    530                 535                 540
Asn Ile Gly Asp Gln Arg Val Tyr Ala Ser Asp Ala Ala His Ala Arg
545                 550                 555                 560
Trp Trp Gln Val Tyr Asp Asp Met Gly Glu Ala Met Arg Ser Pro Cys
                565                 570                 575
Arg Leu Ser Glu Arg Arg Lys Ile Glu Ile Ala Trp Asp Arg His Leu
            580                 585                 590
```

```
Ala Glu Glu Ala Gly Phe Ser Asp His His Trp Lys Ile Asn Ile Thr
        595                 600                 605
Asp Pro Arg Lys Trp Glu
    610
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<210> 27
<211> 626
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (133)..(624)

<400> 27

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ttcgagcggc cgccccgggc aggtacaaac ctgacgtgaa ggctctaaag gagaagctca 60

ggctgcctgt tggttcctgt gagcttgctg ttccactcaa cgcaaaagca cgactcttac 120

acggtagaca ga cgc aga gaa gca tat gct aca ata ctt cat tca gca agt 171
             Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser
               1               5                  10

gaa tat gtt tgc ggt gcg ata aca gca gct caa agc att cgt caa gca   219
Glu Tyr Val Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala
     15                  20                  25

gga tca aca aga gac ctt gtt att ctt gtt gat gac acc ata agt gac   267
Gly Ser Thr Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp
 30                  35                  40                  45

cac cac cgc aag ggg ctg gaa tct gct ggg tgg aag gtc aga ata ata   315
His His Arg Lys Gly Leu Glu Ser Ala Gly Trp Lys Val Arg Ile Ile
                 50                  55                  60

gaa agg atc cgg aat ccc aaa gcc gaa cgt gat gcc tac aac gaa tgg   363
Glu Arg Ile Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp
             65                  70                  75

aac tac agc aaa ttc cgg ctg tgg cag ctt aca gat tac gac aag gtt   411
Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val
         80                  85                  90

att ttc att gat gct gat ctg ctc atc ctg agg aac att gat ttc ttg   459
Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu
     95                  100                 105

ttt gca atg cca gaa atc acc gca act ggg aac aat gcc aca ctc ttc   507
Phe Ala Met Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe
110                 115                 120                 125
```

```
aac tct ggg gtg atg gtc att gaa cct tca aac tgc acg ttc cag tta   555
Asn Ser Gly Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Gln Leu
                130                 135                 140

ctg atg gag cac atc aac gag ata aca tct tac aac ggt ggt gac caa   603
Leu Met Glu His Ile Asn Glu Ile Thr Ser Tyr Asn Gly Gly Asp Gln
            145                 150                 155

ggg tac ctc ggc cgc gac cac gc                                    626
Gly Tyr Leu Gly Arg Asp His
        160
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<210> 28
<211> 164
<212> PRT
<213> Zea mays

<400> 28

```
Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser Glu Tyr Val
  1               5                  10                  15

Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala Gly Ser Thr
             20                  25                  30

Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp His His Arg
         35                  40                  45

Lys Gly Leu Glu Ser Ala Gly Trp Lys Val Arg Ile Ile Glu Arg Ile
     50                  55                  60

Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp Asn Tyr Ser
 65                  70                  75                  80

Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val Ile Phe Ile
                85                  90                  95

Asp Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu Phe Ala Met
            100                 105                 110

Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe Asn Ser Gly
        115                 120                 125

Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Gln Leu Leu Met Glu
    130                 135                 140

His Ile Asn Glu Ile Thr Ser Tyr Asn Gly Gly Asp Gln Gly Tyr Leu
145                 150                 155                 160

Gly Arg Asp His
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<210> 29
<211> 553
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)..(552)

<400> 29

```
tgg aag gtc aga ata ata gaa agg atc cgg aat ccc aaa gcc gaa cgt   48
Trp Lys Val Arg Ile Ile Glu Arg Ile Arg Asn Pro Lys Ala Glu Arg
  1               5                  10                  15

gat gcc tac aac gaa tgg aac tac agc aaa ttc cgg ctg tgg cag ctt   96
Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu
             20                  25                  30

aca gat tac gac aag gtt att ttc att gat gct gat ctg ctc atc ctg   144
Thr Asp Tyr Asp Lys Val Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu
         35                  40                  45

agg aac att gat ttc ttg ttt gca atg cca gaa atc acc gca act ggg   192
Arg Asn Ile Asp Phe Leu Phe Ala Met Pro Glu Ile Thr Ala Thr Gly
     50                  55                  60

aac aat gcc aca ctc ttc aac tct ggg gtg atg gtc att gaa cct tca   240
Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met Val Ile Glu Pro Ser
 65                  70                  75                  80

aac tgc acg ttc cag tta ctg atg gag cac atc aac gag ata aca tct   288
Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile Asn Glu Ile Thr Ser
                 85                  90                  95

tac aac ggt ggt gac caa ggg tac ctg aac gag ata ttc aca tgg tgg   336
Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp Trp
            100                 105                 110

cac cgg att cca aag cac atg aat ttc ttg aag cat ttc tgg gag ggt   384
His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu Gly
        115                 120                 125

gat gag gac gaa gtg aag gcc aag aag act cgg ctg ttc ggc gcc aac   432
Asp Glu Asp Glu Val Lys Ala Lys Lys Thr Arg Leu Phe Gly Ala Asn
    130                 135                 140

cca ccg atc ctc tac gtt ctc cac tac ttg ggg cgg aag cca tgg ctg   480
Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Arg Lys Pro Trp Leu
145                 150                 155                 160
```

```
tgc ttc cgg gac tac gat tgc aac tgg aac gtc gag atc ttg cgg gag   528
Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Glu Ile Leu Arg Glu
                165                 170                 175

ttt gcg agt gac gtt gcg cat gcc c                                 553
Phe Ala Ser Asp Val Ala His Ala
            180
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<210> 30
<211> 184
<212> PRT
<213> Zea mays

<400> 30

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Trp Lys Val Arg Ile Ile Glu Arg Ile Arg Asn Pro Lys Ala Glu Arg
  1               5                  10                  15

Asp Ala Tyr Asn Glu Trp Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu
             20                  25                  30

Thr Asp Tyr Asp Lys Val Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu
         35                  40                  45

Arg Asn Ile Asp Phe Leu Phe Ala Met Pro Glu Ile Thr Ala Thr Gly
     50                  55                  60

Asn Asn Ala Thr Leu Phe Asn Ser Gly Val Met Val Ile Glu Pro Ser
 65                  70                  75                  80

Asn Cys Thr Phe Gln Leu Leu Met Glu His Ile Asn Glu Ile Thr Ser
                85                  90                  95

Tyr Asn Gly Gly Asp Gln Gly Tyr Leu Asn Glu Ile Phe Thr Trp Trp
            100                 105                 110

His Arg Ile Pro Lys His Met Asn Phe Leu Lys His Phe Trp Glu Gly
        115                 120                 125

Asp Glu Asp Glu Val Lys Ala Lys Lys Thr Arg Leu Phe Gly Ala Asn
    130                 135                 140

Pro Pro Ile Leu Tyr Val Leu His Tyr Leu Gly Arg Lys Pro Trp Leu
145                 150                 155                 160
Cys Phe Arg Asp Tyr Asp Cys Asn Trp Asn Val Glu Ile Leu Arg Glu
                165                 170                 175

Phe Ala Ser Asp Val Ala His Ala
            180
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<210> 31
<211> 552
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)..(552)

<400> 31

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tcc ctg cgc cgg ctc agc ccc aac gcc gac cgc gtc gtc atc gcg tcc   48
Ser Leu Arg Arg Leu Ser Pro Asn Ala Asp Arg Val Val Ile Ala Ser
  1               5                  10                  15

ctc gac gtc ccg ccg ctc tgg gtt cag gca ctg aaa aat gac ggg gta   96
Leu Asp Val Pro Pro Leu Trp Val Gln Ala Leu Lys Asn Asp Gly Val
             20                  25                  30

aag gtg gtc tct gtg gag aat ttg aaa aat cct tac gag aaa caa gaa   144
Lys Val Val Ser Val Glu Asn Leu Lys Asn Pro Tyr Glu Lys Gln Glu
         35                  40                  45

aat ttc aac aga cga ttc aaa ttg act tta aac aag ctg tat gca tgg   192
Asn Phe Asn Arg Arg Phe Lys Leu Thr Leu Asn Lys Leu Tyr Ala Trp
     50                  55                  60

agc ttg gtt tca tat gag cga gtt gtt atg ctt gac tct gac aac att   240
Ser Leu Val Ser Tyr Glu Arg Val Val Met Leu Asp Ser Asp Asn Ile
 65                  70                  75                  80

ttc ctc caa aat act gat gag tta ttt cag tgt ggt cag ttc tgt gct   288
Phe Leu Gln Asn Thr Asp Glu Leu Phe Gln Cys Gly Gln Phe Cys Ala
                 85                  90                  95

gtc ttc atc aat ccc tgt atc ttc cat aca ggt ctt ttt gtg ctt cag   336
Val Phe Ile Asn Pro Cys Ile Phe His Thr Gly Leu Phe Val Leu Gln
            100                 105                 110

ccc tca atg gat gtt ttt aag aac atg cta cat gag cta gcg gtt gga   384
Pro Ser Met Asp Val Phe Lys Asn Met Leu His Glu Leu Ala Val Gly
        115                 120                 125

cgt gaa aac cca gat ggg gca gac caa ggc ttc ctt gct agt tat ttc   432
Arg Glu Asn Pro Asp Gly Ala Asp Gln Gly Phe Leu Ala Ser Tyr Phe
    130                 135                 140

ccg gac ttg ctt gat cag cca atg ttc cat cca cca gct aat ggt aca   480
Pro Asp Leu Leu Asp Gln Pro Met Phe His Pro Pro Ala Asn Gly Thr
145                 150                 155                 160

aaa ctt tgg ggt act tat cgc ctc ccc cta ggc tac cag atg gat gca   528
```

Lys Leu Trp Gly Thr Tyr Arg Leu Pro Leu Gly Tyr Gln Met Asp Ala
165 170 175

tct tac tat tat ctg aag ctt cgc 552
Ser Tyr Tyr Tyr Leu Lys Leu Arg
180

<210> 32
<211> 184
<212> PRT
<213> Zea mays

<400> 32
Ser Leu Arg Arg Leu Ser Pro Asn Ala Asp Arg Val Val Ile Ala Ser
1 5 10 15

Leu Asp Val Pro Pro Leu Trp Val Gln Ala Leu Lys Asn Asp Gly Val
20 25 30

Lys Val Val Ser Val Glu Asn Leu Lys Asn Pro Tyr Glu Lys Gln Glu
35 40 45

Asn Phe Asn Arg Arg Phe Lys Leu Thr Leu Asn Lys Leu Tyr Ala Trp
50 55 60

Ser Leu Val Ser Tyr Glu Arg Val Val Met Leu Asp Ser Asp Asn Ile
65 70 75 80

Phe Leu Gln Asn Thr Asp Glu Leu Phe Gln Cys Gly Gln Phe Cys Ala
85 90 95

Val Phe Ile Asn Pro Cys Ile Phe His Thr Gly Leu Phe Val Leu Gln
100 105 110

Pro Ser Met Asp Val Phe Lys Asn Met Leu His Glu Leu Ala Val Gly
115 120 125

Arg Glu Asn Pro Asp Gly Ala Asp Gln Gly Phe Leu Ala Ser Tyr Phe
130 135 140

Pro Asp Leu Leu Asp Gln Pro Met Phe His Pro Pro Ala Asn Gly Thr
145 150 155 160

Lys Leu Trp Gly Thr Tyr Arg Leu Pro Leu Gly Tyr Gln Met Asp Ala
165 170 175

Ser Tyr Tyr Tyr Leu Lys Leu Arg
180

<210> 33
<211> 560
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (1)..(558)

<400> 33

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aaa cct gac gtg aag gcg ttg aag gag aag ctc agg ctg cct gtt ggt   48
Lys Pro Asp Val Lys Ala Leu Lys Glu Lys Leu Arg Leu Pro Val Gly
  1               5                  10                  15

tcc tgt gag ctt gct gtt cca ctc aac gca aaa gca cga ctc tac aca   96
Ser Cys Glu Leu Ala Val Pro Leu Asn Ala Lys Ala Arg Leu Tyr Thr
            20                  25                  30

gta gac aga cgc aga gaa gca tat gcg aca ata ctg cat tca gca agt   144
Val Asp Arg Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser
         35                  40                  45

gaa tat gtt tgc ggc gcg atc acg gca gct caa agc att cgt caa gca   192
Glu Tyr Val Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala
     50                  55                  60

gga tca aca aga gac ctc gtt att ctc gtc gac gac acc ata agt gac   240
Gly Ser Thr Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp
 65                  70                  75                  80

cac cac cgc aag ggg ctg caa tct gcg ggg tgg aag gtc agg ata ata   288
His His Arg Lys Gly Leu Gln Ser Ala Gly Trp Lys Val Arg Ile Ile
                85                  90                  95

cag agg atc cgg aac ccc aaa gcc gag cgc gac gcc tac aac gag tgg   336
Gln Arg Ile Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp
            100                 105                 110

aac tac agc aaa ttc cgg ctg tgg cag ctc acg gat tac gac aag gtc   384
Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val
        115                 120                 125

atc ttc atc gac gcg gat ctc ctc atc ctg agg aac atc gat ttc ctg   432
Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu
    130                 135                 140

ttc gcg ctg ccg gag atc acg gcg acg ggg aac aac gcg acg ctc ttc   480
Phe Ala Leu Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe
145                 150                 155                 160

aac tcg gga gtg atg gtc atc gag cct tcg aac tgc acg ttc cgg cta   528
Asn Ser Gly Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Arg Leu
```

165 170 175

ctg atg gag cac atc gac gag ata acg tcg ta 560
Leu Met Glu His Ile Asp Glu Ile Thr Ser
180 185

<210> 34
<211> 186
<212> PRT
<213> Zea mays

<400> 34
Lys Pro Asp Val Lys Ala Leu Lys Glu Lys Leu Arg Leu Pro Val Gly
1 5 10 15

Ser Cys Glu Leu Ala Val Pro Leu Asn Ala Lys Ala Arg Leu Tyr Thr
20 25 30

Val Asp Arg Arg Arg Glu Ala Tyr Ala Thr Ile Leu His Ser Ala Ser
35 40 45

Glu Tyr Val Cys Gly Ala Ile Thr Ala Ala Gln Ser Ile Arg Gln Ala
50 55 60

Gly Ser Thr Arg Asp Leu Val Ile Leu Val Asp Asp Thr Ile Ser Asp
65 70 75 80

His His Arg Lys Gly Leu Gln Ser Ala Gly Trp Lys Val Arg Ile Ile
85 90 95

Gln Arg Ile Arg Asn Pro Lys Ala Glu Arg Asp Ala Tyr Asn Glu Trp
100 105 110

Asn Tyr Ser Lys Phe Arg Leu Trp Gln Leu Thr Asp Tyr Asp Lys Val
115 120 125

Ile Phe Ile Asp Ala Asp Leu Leu Ile Leu Arg Asn Ile Asp Phe Leu
130 135 140

Phe Ala Leu Pro Glu Ile Thr Ala Thr Gly Asn Asn Ala Thr Leu Phe
145 150 155 160

Asn Ser Gly Val Met Val Ile Glu Pro Ser Asn Cys Thr Phe Arg Leu
165 170 175

Leu Met Glu His Ile Asp Glu Ile Thr Ser
180 185

<210> 35
<211> 566

<212> PRT
<213> Arabidopsis thaliana

<400> 35
Met Gly Ala Lys Ser Lys Ser Ser Ser Thr Arg Phe Phe Met Phe Tyr
 1   5   10   15

Leu Ile Leu Ile Ser Leu Ser Phe Leu Gly Leu Leu Leu Asn Phe Lys
            20   25   30

Pro Leu Phe Leu Leu Asn Pro Met Ile Ala Ser Pro Ser Ile Val Glu
        35   40   45

Ile Arg Tyr Ser Leu Pro Glu Pro Val Lys Arg Thr Pro Ile Trp Leu
    50   55   60

Arg Leu Ile Arg Asn Tyr Leu Pro Asp Glu Lys Lys Ile Arg Val Gly
65   70   75   80

Leu Leu Asn Ile Ala Glu Asn Glu Arg Glu Ser Tyr Glu Ala Ser Gly
                85   90   95

Thr Ser Ile Leu Glu Asn Val His Val Ser Leu Asp Pro Leu Pro Asn
            100   105   110

Asn Leu Thr Trp Thr Ser Leu Phe Pro Val Trp Ile Asp Glu Asp His
        115   120   125

Thr Trp His Ile Pro Ser Cys Pro Glu Val Pro Leu Pro Lys Met Glu
    130   135   140

Gly Ser Glu Ala Asp Val Asp Val Val Val Val Lys Val Pro Cys Asp
145   150   155   160

Gly Phe Ser Glu Lys Arg Gly Leu Arg Asp Val Phe Arg Leu Gln Val
                165   170   175

Asn Leu Ala Ala Ala Asn Leu Val Val Glu Ser Gly Arg Arg Asn Val
            180   185   190

Asp Arg Thr Val Tyr Val Val Phe Ile Gly Ser Cys Gly Pro Met His

        195   200   205
Glu Ile Phe Arg Cys Asp Glu Arg Val Lys Arg Val Gly Asp Tyr Trp
    210   215   220

Val Tyr Arg Pro Asp Leu Thr Arg Leu Lys Gln Lys Leu Leu Met Pro
225   230   235   240

Pro Gly Ser Cys Gln Ile Ala Pro Leu Gly Gln Gly Glu Ala Trp Ile
                245   250   255

```
Gln Asp Lys Asn Arg Asn Leu Thr Ser Glu Lys Thr Thr Leu Ser Ser
           260                 265                 270

Phe Thr Ala Gln Arg Val Ala Tyr Val Thr Leu Leu His Ser Ser Glu
       275                 280                 285

Val Tyr Val Cys Gly Ala Ile Ala Leu Ala Gln Ser Ile Arg Gln Ser
   290                 295                 300

Gly Ser Thr Lys Asp Met Ile Leu Leu His Asp Asp Ser Ile Thr Asn
305                 310                 315                 320

Ile Ser Leu Ile Gly Leu Ser Leu Ala Gly Trp Lys Leu Arg Arg Val
               325                 330                 335

Glu Arg Ile Arg Ser Pro Phe Ser Lys Lys Arg Ser Tyr Asn Glu Trp
           340                 345                 350

Asn Tyr Ser Lys Leu Arg Val Trp Gln Val Thr Asp Tyr Asp Lys Leu
       355                 360                 365

Val Phe Ile Asp Ala Asp Phe Ile Ile Val Lys Asn Ile Asp Tyr Leu
   370                 375                 380

Phe Ser Tyr Pro Gln Leu Ser Ala Ala Gly Asn Asn Lys Val Leu Phe
385                 390                 395                 400

Asn Ser Gly Val Met Val Leu Glu Pro Ser Ala Cys Leu Phe Glu Asp
               405                 410                 415

Leu Met Leu Lys Ser Phe Lys Ile Gly Ser Tyr Asn Gly Gly Asp Gln
           420                 425                 430

Gly Phe Leu Asn Glu Tyr Phe Val Trp Trp His Arg Leu Ser Lys Arg
       435                 440                 445

Leu Asn Thr Met Lys Tyr Phe Gly Asp Glu Ser Arg His Asp Lys Ala
   450                 455                 460

Arg Asn Leu Pro Glu Asn Leu Glu Gly Ile His Tyr Leu Gly Leu Lys
465                 470                 475                 480

Pro Trp Arg Cys Tyr Arg Asp Tyr Asp Cys Asn Trp Asp Leu Lys Thr
               485                 490                 495

Arg Arg Val Tyr Ala Ser Glu Ser Val His Ala Arg Trp Trp Lys Val
           500                 505                 510

Tyr Asp Lys Met Pro Lys Lys Leu Lys Gly Tyr Cys Gly Leu Asn Leu
       515                 520                 525

Lys Met Glu Lys Asn Val Glu Lys Trp Arg Lys Met Ala Lys Leu Asn
```

530 535 540

Gly Phe Pro Glu Asn His Trp Lys Ile Arg Ile Lys Asp Pro Arg Lys
545 550 555 560

Lys Asn Arg Leu Ser Glu
565